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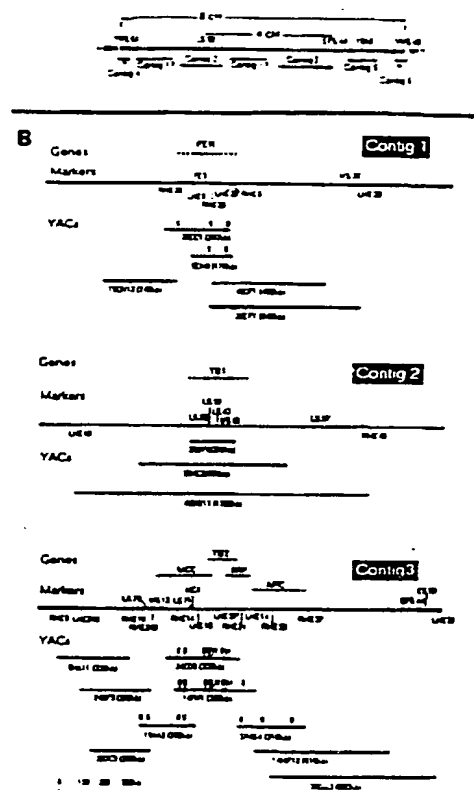
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## (57) Abstract

A human gene termed APC is disclosed. Methods and kits are provided for assessing mutations of the APC gene in human tissues and body samples. APC mutations are found in familial adenomatous polyposis patients as well as in sporadic colorectal cancer patients. APC is expressed in most normal tissues. These results suggest that APC is a tumor suppressor.



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## INHERITED AND SOMATIC MUTATIONS OF APC GENE IN COLORECTAL CANCER OF HUMANS

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### TECHNICAL AREA OF THE INVENTION

The invention relates to the area of cancer diagnostics and therapeutics. More particularly, the invention relates to detection of the germline and somatic alterations of wild-type APC genes. In addition, it relates to therapeutic intervention to restore the function of APC gene product.

### BACKGROUND OF THE INVENTION

According to the model of Knudson for tumorigenesis (Cancer Research, Vol. 45, p. 1482, 1985), there are tumor suppressor genes in all normal cells which, when they become non-functional due to mutation, cause neoplastic development. Evidence for this model has been found in the cases of retinoblastoma and colorectal tumors. The implicated suppressor genes in those tumors, RB, p53, DCC and MCC, were found to be deleted or altered in many cases of the tumors studied. (Hansen and Cavenee, Cancer Research, Vol. 47, pp. 5518-5527 (1987); Baker et al., Science, Vol. 244, p. 217 (1989); Fearon et al., Science, Vol. 247, p. 49 (1990); Kinzler et al. Science Vol. 251, p. 1366 (1991).)

In order to fully understand the pathogenesis of tumors, it will be necessary to identify the other suppressor genes that play a role in the tumorigenesis process. Prominent among these is the one(s) presumptively located at 5q21. Cytogenetic (Herrera et al., Am J. Med. Genet., Vol. 25, p. 473 (1986) and linkage (Leppert et al., Science, Vol. 238, p. 1411 (1987); Bodmer et al., Nature, Vol. 328, p. 614 (1987)) studies have shown that this chromosome region harbors the gene

responsible for familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS). FAP is an autosomal-dominant, inherited disease in which affected individuals develop hundreds to thousands of adenomatous polyps, some of which progress to malignancy. GS is a variant of FAP in which desmoid tumors, osteomas and other soft tissue tumors occur together with multiple adenomas of the colon and rectum. A less severe form of polyposis has been identified in which only a few (2-40) polyps develop. This condition also is familial and is linked to the same chromosomal markers as FAP and GS (Leppert et al., New England Journal of Medicine, Vol. 322, pp. 904-908, 1990.) Additionally, this chromosomal region is often deleted from the adenomas (Vogelstein et al., N. Engl. J. Med., Vol. 319, p. 525 (1988)) and carcinomas (Vogelstein et al., N. Engl. J. Med., Vol. 319, p. 525 (1988); Solomon et al., Nature, Vol. 328, p. 616 (1987); Sasaki et al., Cancer Research, Vol. 49, p. 4402 (1989); Delattre et al., Lancet, Vol. 2, p. 353 (1989); and Ashton-Rickardt et al., Oncogene, Vol. 4, p. 1169 (1989)) of patients without FAP (sporadic tumors). Thus, a putative suppressor gene on chromosome 5q21 appears to play a role in the early stages of colorectal neoplasia in both sporadic and familial tumors.

Although the MCC gene has been identified on 5q21 as a candidate suppressor gene, it does not appear to be altered in FAP or GS patients. Thus there is a need in the art for investigations of this chromosomal region to identify genes and to determine if any of such genes are associated with FAP and/or GS and the process of tumorigenesis.

#### **SUMMARY OF THE INVENTION**

It is an object of the present invention to provide a method for diagnosing and prognosing a neoplastic tissue of a human.

It is another object of the invention to provide a method of detecting genetic predisposition to cancer.

It is another object of the invention to provide a method of supplying wild-type APC gene function to a cell which has lost said gene function.

It is yet another object of the invention to provide a kit for determination of the nucleotide sequence of APC alleles by the polymerase chain reaction.

It is still another object of the invention to provide nucleic acid probes for detection of mutations in the human APC gene.

It is still another object of the invention to provide a cDNA molecule encoding the APC gene product.

It is yet another object of the invention to provide a preparation of the human APC protein.

It is another object of the invention to provide a method of screening for genetic predisposition to cancer.

It is an object of the invention to provide methods of testing therapeutic agents for the ability to suppress neoplasia.

It is still another object of the invention to provide animals carrying mutant APC alleles.

These and other objects of the invention are provided by one or more of the embodiments which are described below. In one embodiment of the present invention a method of diagnosing or prognosing a neoplastic tissue of a human is provided comprising: detecting somatic alteration of wild-type APC genes or their expression products in a sporadic colorectal cancer tissue, said alteration indicating neoplasia of the tissue.

In yet another embodiment a method is provided of detecting genetic predisposition to cancer in a human including familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS), comprising: isolating a human sample selected from the group consisting of blood and fetal tissue; detecting alteration of wild-type APC gene coding sequences or their expression products from the sample, said alteration indicating genetic predisposition to cancer.

In another embodiment of the present invention a method is provided for supplying wild-type APC gene function to a cell which has lost said gene function by virtue of a mutation in the APC gene, comprising: introducing a wild-type APC gene into a cell which has lost said gene function such that said wild-type gene is expressed in the cell.

In another embodiment a method of supplying wild-type APC gene function to a cell is provided comprising: introducing a portion of a wild-type APC gene into a cell which has lost said gene function such

that said portion is expressed in the cell, said portion encoding a part of the APC protein which is required for non-neoplastic growth of said cell. APC protein can also be applied to cells or administered to animals to remediate for mutant APC genes. Synthetic peptides or drugs can also be used to mimic APC function in cells which have altered APC expression.

In yet another embodiment a pair of single stranded primers is provided for determination of the nucleotide sequence of the APC gene by polymerase chain reaction. The sequence of said pair of single stranded DNA primers is derived from chromosome 5q band 21, said pair of primers allowing synthesis of APC gene coding sequences.

In still another embodiment of the invention a nucleic acid probe is provided which is complementary to human wild-type APC gene coding sequences and which can form mismatches with mutant APC genes, thereby allowing their detection by enzymatic or chemical cleavage or by shifts in electrophoretic mobility.

In another embodiment of the invention a method is provided for detecting the presence of a neoplastic tissue in a human. The method comprises isolating a body sample from a human; detecting in said sample alteration of a wild-type APC gene sequence or wild-type APC expression product, said alteration indicating the presence of a neoplastic tissue in the human.

In still another embodiment a cDNA molecule is provided which comprises the coding sequence of the APC gene.

In even another embodiment a preparation of the human APC protein is provided which is substantially free of other human proteins. The amino acid sequence of the protein is shown in Figure 3 or 7.

In yet another embodiment of the invention a method is provided for screening for genetic predisposition to cancer, including familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS), in a human. The method comprises: detecting among kindred persons the presence of a DNA polymorphism which is linked to a mutant APC allele in an individual having a genetic predisposition to cancer, said kindred being genetically related to the individual, the presence of said polymorphism suggesting a predisposition to cancer.



In another embodiment of the invention a method of testing therapeutic agents for the ability to suppress a neoplastically transformed phenotype is provided. The method comprises: applying a test substance to a cultured epithelial cell which carries a mutation in an APC allele; and determining whether said test substance suppresses the neoplastically transformed phenotype of the cell.

In another embodiment of the invention a method of testing therapeutic agents for the ability to suppress a neoplastically transformed phenotype is provided. The method comprises: administering a test substance to an animal which carries a mutant APC allele; and determining whether said test substance prevents or suppresses the growth of tumors.

In still other embodiments of the invention transgenic animals are provided. The animals carry a mutant APC allele from a second animal species or have been genetically engineered to contain an insertion mutation which disrupts an APC allele.

The present invention provides the art with the information that the APC gene, a heretofore unknown gene is, in fact, a target of mutational alterations on chromosome 5q21 and that these alterations are associated with the process of tumorigenesis. This information allows highly specific assays to be performed to assess the neoplastic status of a particular tissue or the predisposition to cancer of an individual. This invention has applicability to Familial Adenomatous Polyposis, sporadic colorectal cancers, Gardner's Syndrome, as well as the less severe familial polyposis discusses above.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1A shows an overview of yeast artificial chromosome (YAC) contigs. Genetic distances between selected RFLP markers from within the contigs are shown in centiMorgans.

Figure 1B shows a detailed map of the three central contigs. The position of the six identified genes from within the FAP region is shown; the 5' and 3' ends of the transcripts from these genes have in general not yet been isolated, as indicated by the string of dots surrounding the bars denoting the genes' positions. Selected restriction

endonuclease recognition sites are indicated. B, BssH2; S, SstII; M, MluI; N, NruI.

Figure 2 shows the sequence of TB1 and TB2 genes. The cDNA sequence of the TB1 gene was determined from the analysis of 11 cDNA clones derived from normal colon and liver, as described in the text. A total of 2314 bp were contained within the overlapping cDNA clones, defining an ORF of 424 amino acids beginning at nucleotide 1. Only the predicted amino acids from the ORF are shown. The carboxy-terminal end of the ORF has apparently been identified, but the 5' end of the TB1 transcript has not yet been precisely determined.

The cDNA sequence of the TB2 gene was determined from the YS-39 clone derived as described in the text. This clone consisted of 2300 bp and defined an ORF of 185 amino acids beginning at nucleotide 1. Only the predicted amino acids are shown. The carboxy terminal end of the ORF has apparently been identified, but the 5' end of the TB2 transcript has not been precisely determined.

Figure 3 shows the sequence of the APC gene product. The cDNA sequence was determined through the analysis of 87 cDNA clones derived from normal colon, liver, and brain. A total of 8973 bp were contained within overlapping cDNA clones, defining an ORF of 2842 amino acids. In frame stop codons surrounded this ORF, as described in the text, suggesting that the entire APC gene product was represented in the ORF illustrated. Only the predicted amino acids are shown.

Figure 4 shows the local similarity between human APC and ral2 of yeast. Local similarity among the APC and MCC genes and the m3 muscarinic acetylcholine receptor is shown. The region of the mAChR shown corresponds to that responsible for coupling the receptor to G proteins. The connecting lines indicate identities; dots indicate related amino acids residues.

Figure 5 shows the genomic map of the 1200 kb NotI fragment at the FAP locus. The NotI fragment is shown as a bold line. Relevant parts of the deletion chromosomes from patients 3214 and 3824 are shown as stippled lines. Probes used to characterize the NotI fragment and the deletions, and three YACs from which subclones were obtained, are shown below the restriction map. The chimeric end of YAC

183H12 is indicated by a dotted line. The orientation and approximate position of MCC are indicated above the map.

Figure 6 shows the DNA sequence and predicted amino acid sequence of DP1 (TB2). The nucleotide numbering begins at the most 5' nucleotide isolated. A proposed initiation methionine (base 77) is indicated in bold type. The entire coding sequence is presented.

Figure 7 shows the cDNA and predicted amino acid sequence of DP2.5 (APC). The nucleotide numbering begins at the proposed initiation methionine. The nucleotides and amino acids of the alternatively spliced exon (exon 9; nucleotide positions 934-1236) are presented in lower case letters. At the 3' end, a poly(A) addition signal occurs at 9530, and one cDNA clone has a poly(A) at 9563. Other cDNA clones extend beyond 9563, however, and their consensus sequence is included here.

Figure 8 shows the arrangement of exons in DP2.5 (APC). (A) Exon 9 corresponds to nucleotides 933-1312; exon 9a corresponds to nucleotides 1236-1312. The stop codon in the cDNA is at nucleotide 8535. (B) Partial intronic sequence surrounding each exon is shown.

#### DETAILED DESCRIPTION

It is a discovery of the present invention that mutational events associated with tumorigenesis occur in a previously unknown gene on chromosome 5q named here the APC (Adenomatous Polyposis Coli) gene. Although it was previously known that deletion of alleles on chromosome 5q were common in certain types of cancers, it was not known that a target gene of these deletions was the APC gene. Further it was not known that other types of mutational events in the APC gene are also associated with cancers. The mutations of the APC gene can involve gross rearrangements, such as insertions and deletions. Point mutations have also been observed.

According to the diagnostic and prognostic method of the present invention, alteration of the wild-type APC gene is detected. "Alteration of a wild-type gene" according to the present invention encompasses all forms of mutations — including deletions. The alteration may be due to either rearrangements such as insertions, inversions, and deletions, or to point mutations. Deletions may be of the

entire gene or only a portion of the gene. Somatic mutations are those which occur only in certain tissues, e.g., in the tumor tissue, and are not inherited in the germline. Germline mutations can be found in any of a body's tissues. If only a single allele is somatically mutated, an early neoplastic state is indicated. However, if both alleles are mutated then a late neoplastic state is indicated. The finding of APC mutations thus provides both diagnostic and prognostic information. An APC allele which is not deleted (e.g., that on the sister chromosome to a chromosome carrying an APC deletion) can be screened for other mutations, such as insertions, small deletions, and point mutations. It is believed that many mutations found in tumor tissues will be those leading to decreased expression of the APC gene product. However, mutations leading to non-functional gene products would also lead to a cancerous state. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the APC gene product.

In order to detect the alteration of the wild-type APC gene in a tissue, it is helpful to isolate the tissue free from surrounding normal tissues. Means for enriching a tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometry. These as well as other techniques for separating tumor from normal cells are well known in the art. If the tumor tissue is highly contaminated with normal cells, detection of mutations is more difficult.

Detection of point mutations may be accomplished by molecular cloning of the APC allele (or alleles) and sequencing that allele(s) using techniques well known in the art. Alternatively, the polymerase chain reaction (PCR) can be used to amplify gene sequences directly from a genomic DNA preparation from the tumor tissue. The DNA sequence of the amplified sequences can then be determined. The polymerase chain reaction itself is well known in the art. See, e.g., Saiki et al., Science, Vol. 239, p. 487, 1988; U.S. 4,683,203; and U.S. 4,683,195.

Specific primers which can be used in order to amplify the gene will be discussed in more detail below. The ligase chain reaction, which is known in the art, can also be used to amplify APC sequences. See Wu et al., Genomics, Vol. 4, pp. 560-569 (1989). In addition, a technique known as allele specific PCR can be used. (See Ruano and Kidd, Nucleic Acids Research, Vol. 17, p. 8392, 1989.) According to this technique, primers are used which hybridize at their 3' ends to a particular APC mutation. If the particular APC mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., Nucleic Acids Research, Vol. 17, p.7, 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening among kindred persons of an affected individual for the presence of the APC mutation found in that individual. Single stranded conformation polymorphism (SSCP) analysis can also be used to detect base change variants of an allele. (Orita et al., Proc. Natl. Acad. Sci. USA Vol. 86, pp. 2766-2770, 1989, and Genomics, Vol. 5, pp. 874-879, 1989.) Other techniques for detecting insertions and deletions as are known in the art can be used.

Alteration of wild-type genes can also be detected on the basis of the alteration of a wild-type expression product of the gene. Such expression products include both the APC mRNA as well as the APC protein product. The sequences of these products are shown in Figures 3 and 7. Point mutations may be detected by amplifying and sequencing the mRNA or via molecular cloning of cDNA made from the mRNA. The sequence of the cloned cDNA can be determined using DNA sequencing techniques which are well known in the art. The cDNA can also be sequenced via the polymerase chain reaction (PCR) which will be discussed in more detail below.

Mismatches, according to the present invention are hybridized nucleic acid duplexes which are not 100% homologous. The lack of

total homology may be due to deletions, insertions, inversions, substitutions or frameshift mutations. Mismatch detection can be used to detect point mutations in the gene or its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of tumor samples. An example of a mismatch cleavage technique is the RNase protection method, which is described in detail in Winter et al., Proc. Natl. Acad. Sci. USA, Vol. 82, p. 7575, 1985 and Meyers et al., Science, Vol. 230, p. 1242, 1985. In the practice of the present invention the method involves the use of a labeled riboprobe which is complementary to the human wild-type APC gene coding sequence. The riboprobe and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full-length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the APC mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the APC mRNA or gene it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., Proc. Natl. Acad. Sci. USA, Vol. 85, 4397, 1988; and Shenk et al., Proc. Natl. Acad. Sci. USA, Vol. 72, p. 989, 1975. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, Human Genetics, Vol. 42, p. 726, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the APC gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the APC gene which have been amplified by use of polymerase chain reaction may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the APC gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the APC gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the APC gene. Hybridization of allele-specific probes with amplified APC sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under stringent hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the allele-specific probe.

Alteration of APC mRNA expression can be detected by any technique known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type APC gene.

Alteration of wild-type APC genes can also be detected by screening for alteration of wild-type APC protein. For example, monoclonal antibodies immunoreactive with APC can be used to screen a tissue. Lack of cognate antigen would indicate an APC mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant APC gene product. Such immunological assays can be done in any convenient format known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered APC protein can be used to detect alteration of wild-type APC genes. Functional assays can be used, such as protein binding determinations. For example, it is believed that APC protein oligomerizes to itself and/or MCC protein or binds to a G protein. Thus, an assay for the ability to bind to wild type APC or MCC protein or that G protein can be employed. In addition, assays can be used which detect APC biochemical function. It is believed that APC is involved in phospholipid metabolism. Thus, assaying the enzymatic products of the involved phospholipid metabolic pathway can be used to

determine APC activity. Finding a mutant APC gene product indicates alteration of a wild-type APC gene.

Mutant APC genes or gene products can also be detected in other human body samples, such as, serum, stool, urine and sputum. The same techniques discussed above for detection of mutant APC genes or gene products in tissues can be applied to other body samples. Cancer cells are sloughed off from tumors and appear in such body samples. In addition, the APC gene product itself may be secreted into the extracellular space and found in these body samples even in the absence of cancer cells. By screening such body samples, a simple early diagnosis can be achieved for many types of cancers. In addition, the progress of chemotherapy or radiotherapy can be monitored more easily by testing such body samples for mutant APC genes or gene products.

The methods of diagnosis of the present invention are applicable to any tumor in which APC has a role in tumorigenesis. Deletions of chromosome arm 5q have been observed in tumors of lung, breast, colon, rectum, bladder, liver, sarcomas, stomach and prostate, as well as in leukemias and lymphomas. Thus these are likely to be tumors in which APC has a role. The diagnostic method of the present invention is useful for clinicians so that they can decide upon an appropriate course of treatment. For example, a tumor displaying alteration of both APC alleles might suggest a more aggressive therapeutic regimen than a tumor displaying alteration of only one APC allele.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular APC allele using the polymerase chain reaction. The pairs of single stranded DNA primers can be annealed to sequences within or surrounding the APC gene on chromosome 5q in order to prime amplifying DNA synthesis of the APC gene itself. A complete set of these primers allows synthesis of all of the nucleotides of the APC gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele specific primers can also be used. Such primers anneal only to particular APC mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.



In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from APC sequences or sequences adjacent to APC except the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available. Given the sequence of the APC open reading frame shown in Figure 7, design of particular primers is well within the skill of the art.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the APC gene or mRNA using other techniques. Mismatches can be detected using either enzymes (e.g., S1 nuclease), chemicals (e.g., hydroxylamine or osmium tetroxide and piperidine), or changes in electrophoretic mobility of mismatched hybrids as compared to totally matched hybrids. These techniques are known in the art. See, Cotton, supra, Shenk, supra, Myers, supra, Winter, supra, and Novack et al., Proc. Natl. Acad. Sci. USA, Vol. 83, p. 586, 1986. Generally, the probes are complementary to APC gene coding sequences, although probes to certain introns are also contemplated. An entire battery of nucleic acid probes is used to compose a kit for detecting alteration of wild-type APC genes. The kit allows for hybridization to the entire APC gene. The probes may overlap with each other or be contiguous.

If a riboprobe is used to detect mismatches with mRNA, it is complementary to the mRNA of the human wild-type APC gene. The riboprobe thus is an anti-sense probe in that it does not code for the APC protein because it is of the opposite polarity to the sense strand. The riboprobe generally will be labeled with a radioactive, colorimetric, or fluorometric material, which can be accomplished by

any means known in the art. If the riboprobe is used to detect mismatches with DNA it can be of either polarity, sense or anti-sense. Similarly, DNA probes also may be used to detect mismatches.

Nucleic acid probes may also be complementary to mutant alleles of the APC gene. These are useful to detect similar mutations in other patients on the basis of hybridization rather than mismatches. These are discussed above and referred to as allele-specific probes. As mentioned above, the APC probes can also be used in Southern hybridizations to genomic DNA to detect gross chromosomal changes such as deletions and insertions. The probes can also be used to select cDNA clones of APC genes from tumor and normal tissues. In addition, the probes can be used to detect APC mRNA in tissues to determine if expression is diminished as a result of alteration of wild-type APC genes. Provided with the APC coding sequence shown in Figure 7 (SEQ ID NO: 1), design of particular probes is well within the skill of the ordinary artisan.

According to the present invention a method is also provided of supplying wild-type APC function to a cell which carries mutant APC alleles. Supplying such function should suppress neoplastic growth of the recipient cells. The wild-type APC gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation the gene will be expressed by the cell from the extrachromosomal location. If a gene portion is introduced and expressed in a cell carrying a mutant APC allele, the gene portion should encode a part of the APC protein which is required for non-neoplastic growth of the cell. More preferred is the situation where the wild-type APC gene or a part of it is introduced into the mutant cell in such a way that it recombines with the endogenous mutant APC gene present in the cell. Such recombination requires a double recombination event which results in the correction of the APC gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art and the choice of method is

within the competence of the routineer. Cells transformed with the wild-type APC gene can be used as model systems to study cancer remission and drug treatments which promote such remission.

Similarly, cells and animals which carry a mutant APC allele can be used as model systems to study and test for substances which have potential as therapeutic agents. The cells are typically cultured epithelial cells. These may be isolated from individuals with APC mutations, either somatic or germline. Alternatively, the cell line can be engineered to carry the mutation in the APC allele. After a test substance is applied to the cells, the neoplastically transformed phenotype of the cell will be determined. Any trait of neoplastically transformed cells can be assessed, including anchorage-independent growth, tumorigenicity in nude mice, invasiveness of cells, and growth factor dependence. Assays for each of these traits are known in the art.

Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant APC alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous APC gene(s) of the animals may be disrupted by insertion or deletion mutation. After test substances have been administered to the animals, the growth of tumors must be assessed. If the test substance prevents or suppresses the growth of tumors, then the test substance is a candidate therapeutic agent for the treatment of FAP and/or sporadic cancers.

Polypeptides which have APC activity can be supplied to cells which carry mutant or missing APC alleles. The sequence of the APC protein is disclosed in Figure 3 or 7 (SEQ ID NO: 7 or 1). These two sequences differ slightly and appear to indicate the existence of two different forms of the APC protein. Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, APC can be extracted from APC-producing mammalian cells such as brain cells. In addition, the techniques of synthetic chemistry can be employed to synthesize APC protein. Any of such techniques can provide the preparation of the present invention which comprises the APC protein. The preparation

is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or in vitro.

Active APC molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, some such active molecules may be taken up by cells, actively or by diffusion. Extracellular application of APC gene product may be sufficient to affect tumor growth. Supply of molecules with APC activity should lead to a partial reversal of the neoplastic state. Other molecules with APC activity may also be used to effect such a reversal, for example peptides, drugs, or organic compounds.

The present invention also provides a preparation of antibodies immunoreactive with a human APC protein. The antibodies may be polyclonal or monoclonal and may be raised against native APC protein, APC fusion proteins, or mutant APC proteins. The antibodies should be immunoreactive with APC epitopes, preferably epitopes not present on other human proteins. In a preferred embodiment of the invention the antibodies will immunoprecipitate APC proteins from solution as well as react with APC protein on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, the antibodies will detect APC proteins in paraffin or frozen tissue sections, using immunocytochemical techniques. Techniques for raising and purifying antibodies are well known in the art and any such techniques may be chosen to achieve the preparation of the invention.

Predisposition to cancers as in FAP and GS can be ascertained by testing any tissue of a human for mutations of the APC gene. For example, a person who has inherited a germline APC mutation would be prone to develop cancers. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells, or amniotic fluid for mutations of the APC gene. Alteration of a wild-type APC allele, whether for example, by point mutation or by deletion, can be detected by any of the means discussed above.

Molecules of cDNA according to the present invention are intron-free, APC gene coding molecules. They can be made by reverse

transcriptase using the APC mRNA as a template. These molecules can be propagated in vectors and cell lines as is known in the art. Such molecules have the sequence shown in SEQ ID NO: 7. The cDNA can also be made using the techniques of synthetic chemistry given the sequence disclosed herein.

A short region of homology has been identified between APC and the human m3 muscarinic acetylcholine receptor (mAChR). This homology was largely confined to 29 residues in which 6 out of 7 amino acids (EL(GorA)GLQA) were identical (See Figure 4). Initially, it was not known whether this homology was significant, because many other proteins had higher levels of global homology (though few had six out of seven contiguous amino acids in common). However, a study on the sequence elements controlling G protein activation by mAChR subtypes (Lechleiter et al., EMBO J., p. 4381 (1990)) has shown that a 21 amino acid region from the m3 mAChR completely mediated G protein specificity when substituted for the 21 amino acids of m2 mAChR at the analogous protein position. These 21 residues overlap the 19 amino acid homology between APC and m3 mAChR.

This connection between APC and the G protein activating region of mAChR is intriguing in light of previous investigations relating G proteins to cancer. For example, the RAS oncogenes, which are often mutated in colorectal cancers (Vogelstein, et al., N. Engl. J. Med., Vol. 319, p. 525 (1988); Bos et al., Nature Vol. 327, p. 293 (1987)), are members of the G protein family (Bourne, et al., Nature, Vol. 348, p. 125 (1990)) as is an in vitro transformation suppressor (Noda et al., Proc. Natl. Acad. Sci. USA, Vol. 86, p. 162 (1989)) and genes mutated in hormone producing tumors (Candis et al., Nature, Vol. 340, p. 692 (1989); Lyons et al., Science, Vol. 249, p. 655 (1990)). Additionally, the gene responsible for neurofibromatosis (presumably a tumor suppressor gene) has been shown to activate the GTPase activity of RAS (Xu et al., Cell, Vol. 63, p. 835 (1990); Martin et al., Cell, Vol. 63, p. 843 (1990); Ballester et al., Cell, Vol. 63, p. 851 (1990)). Another interesting link between G proteins and colon cancer involves the drug sulindac. This agent has been shown to inhibit the growth of benign colon tumors in patients with FAP, presumably by virtue of its activity as a

cyclooxygenase inhibitor (Waddell et al., J. Surg. Oncology 24(1), 83 (1983); Wadell, et al., Am. J. Surg., 157(1), 175 (1989); Charneau et al., Gastroenterologie Clinique et Biologique 14(2), 153 (1990)). Cyclooxygenase is required to convert arachidonic acid to prostaglandins and other biologically active molecules. G proteins are known to regulate phospholipase A2 activity, which generates arachidonic acid from phospholipids (Role et al., Proc. Natl. Acad. Sci. USA, Vol. 84, p. 3623 (1987); Kurachi et al., Nature, Vol. 337, 12 555 (1989)). Therefore we propose that wild-type APC protein functions by interacting with a G protein and is involved in phospholipid metabolism.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention which has been described in broad terms above.

Example 1:

This example demonstrates the isolation of a 5.5 Mb region of human DNA linked to the FAP locus. Six genes are identified in this region, all of which are expressed in normal colon cells and in colorectal, lung, and bladder tumors.

The cosmid markers YN5.64 and YN5.48 have previously been shown to delimit an 8 cM region containing the locus for FAP (Nakamura et al., Am. J. Hum. Genet. Vol. 43, p. 638 (1988)). Further linkage and pulse-field gel electrophoresis (PFGE) analysis with additional markers has shown that the FAP locus is contained within a 4 cM region bordered by cosmids EF5.44 and L5.99. In order to isolate clones representing a significant portion of this locus, a yeast artificial chromosome (YAC) library was screened with various 5q21 markers. Twenty-one YAC clones, distributed within six contigs and including 5.5 Mb from the region between YN5.64 and YN5.48, were obtained (Figure 1A).

Three contigs encompassing approximately 4Mb were contained within the central portion of this region. The YAC's constituting these contigs, together with the markers used for their isolation and orientations, are shown in Figure 1. These YAC contigs were obtained in the following way. To initiate each contig, the sequence of a genomic

marker cloned from chromosome 5q21 was determined and used to design primers for PCR. PCR was then carried out on pools of YAC clones distributed in microtiter trays as previously described (Anand et al., *Nucleic Acids Research*, Vol. 18, p. 1951 (1980)). Individual YAC clones from the positive pools were identified by further PCR or hybridization based assays, and the YAC sizes were determined by PFGE.

To extend the areas covered by the original YAC clones, "chromosomal walking" was performed. For this purpose, YAC termini were isolated by a PCR based method and sequenced (Riley et al., *Nucleic Acids Research*, Vol. 18, p. 2887 (1990)). PCR primers based on these sequences were then used to rescreen the YAC library. For example, the sequence from an intron of the FER gene (Hao et al., *Mol. Cell. Biol.*, Vol. 9, p. 1587 (1989)) was used to design PCR primers for isolation of the 28EC1 and 5EH8 YACs. The termini of the 28EC1 YAC were sequenced to derive markers RHE28 and LHE28, respectively. The sequences of these two markers were then used to isolate YAC clones 15CH12 (from RHE28) and 40CF1 and 29EF1 (from LHE28). These five YAC's formed a contig encompassing 1200 kb (contig 1, Figure 1B).

Similarly, contig 2 was initiated using cosmid N5.66 sequences, and contig 3 was initiated using sequences both from the MCC gene and from cosmid EF5.44. A walk in the telomeric direction from YAC 14FH1 and a walk in the opposite direction from YAC 39GG3 allowed connection of the initial contig 3 clones through YAC 37HG4 (Figure 1B).

Multipoint linkage analysis with the various markers used to define the contigs, combined with PFGE analysis, showed that contigs 1 and 2 were centromeric to contig 3. These contigs were used as tools to orient and/or identify genes which might be responsible for FAP. Six genes were found to lie within this cluster of YAC's, as follows:

Contig #1: FER - The FER gene was discovered through its homology to the viral oncogene ABL (Hao et al., *supra*). It has an intrinsic tyrosine kinase activity, and in situ hybridization with an FER probe showed that the gene was located at 5q11-23 (Morris et al.,

Cytogenet. Cell. Genet., Vol. 53, p. 4, (1990)). Because of the potential role of this oncogene-related gene in neoplasia, we decided to evaluate it further with regards to the FAP locus. A human genomic clone from FER was isolated (MF 2.3) and used to define a restriction fragment length polymorphism (RFLP), and the RFLP in turn used to map FER by linkage analysis using a panel of three generation families. This showed that FER was very tightly linked to previously defined polymorphic markers for the FAP locus. The genetic mapping of FER was complemented by physical mapping using the YAC clones derived from FER sequences (Figure 1B). Analysis of YAC contig 1 showed that FER was within 600 kb of cosmid marker M5.28, which maps to within 1.5 Mb of cosmid L5.99 by PFGE of human genomic DNA. Thus, the YAC mapping results were consistent with the FER linkage data and PFGE analyses.

Contig 2: TB1 - TB1 was identified through a cross-hybridization approach. Exons of genes are often evolutionarily conserved while introns and intergenic regions are much less conserved. Thus, if a human probe cross-hybridizes strongly to the DNA from non-primate species, there is a reasonable chance that it contains exon sequences. Subclones of the cosmids shown in Figure 1 were used to screen Southern blots containing rodent DNA samples. A subclone of cosmid N5.66 (p 5.66-4) was shown to strongly hybridize to rodent DNA, and this clone was used to screen cDNA libraries derived from normal adult colon and fetal liver. The ends of the initial cDNA clones obtained in this screen were then used to extend the cDNA sequence. Eventually, 11 cDNA clones were isolated, covering 2314 bp. The gene detected by these clones was named TB1. Sequence analysis of the overlapping clones revealed an open reading frame (ORF) that extended for 1302 bp starting from the most 5' sequence data obtained (Figure 2A). If this entire open reading frame were translated, it would encode 434 amino acids. The product of this gene was not globally homologous to any other sequence in the current database but showed two significant local similarities to a family of ADP, ATP carrier/translocator proteins and mitochondrial brown fat uncoupling proteins which are widely distributed from yeast to mammals. These conserved regions of TB1



(underlined in Figure 2A) may define a predictive motif for this sequence family. In addition, TB1 appeared to contain a signal peptide (or mitochondrial targeting sequence) as well as at least 7 transmembrane domains.

Contig 3: MCC, TB2, SRP and APC - The MCC gene was also discovered through a cross-hybridization approach, as described previously (Kinzler et al., Science Vol. 251, p. 1366 (1991)). The MCC gene was considered a candidate for causing FAP by virtue of its tight genetic linkage to FAP susceptibility and its somatic mutation in sporadic colorectal carcinomas. However, mapping experiments suggested that the coding region of MCC was approximately 50 kb proximal to the centromeric end of a 200 kb deletion found in an FAP patient. MCC cDNA probes detected a 10 kb mRNA transcript on Northern blot analysis of which 4151 bp, including the entire open reading frame, have been cloned. Although the 3' non-translated portion or an alternatively spliced form of MCC might have extended into this deletion, it was possible that the deletion did not affect the MCC gene product. We therefore used MCC sequences to initiate a YAC contig, and subsequently used the YAC clones to identify genes 50 to 250 kb distal to MCC that might be contained within the deletion.

In a first approach, the insert from YAC24ED6 (Figure 1B) was radiolabelled and hybridized to a cDNA library from normal colon. One of the cDNA clones (YS39) identified in this manner detected a 3.1 kb mRNA transcript when used as a probe for Northern blot hybridization. Sequence analysis of the YS39 clone revealed that it encompassed 2283 nucleotides and contained an ORF that extended for 555 bp from the most 5' sequence data obtained. If all of this ORF were translated, it would encode 185 amino acids (Figure 2B). The gene detected by YS39 was named TB2. Searches of nucleotide and protein databases revealed that the TB2 gene was not identical to any previously reported sequences nor were there any striking similarities.

Another clone (YS11) identified through the YAC 24ED6 screen appeared to contain portions of two distinct genes. Sequences from one end of YS11 were identical to at least 180 bp of the signal recognition particle protein SRP19 (Lingelbach et al. Nucleic Acids Research,

Vol. 16, p. 9431 (1988). A second ORF, from the opposite end of clone YS11, proved to be identical to 78 bp of a novel gene which was independently identified through a second YAC-based approach. For the latter, DNA from yeast cells containing YAC 14FH1 (Figure 1B) was digested with EcoRI and subcloned into a plasmid vector. Plasmids that contained human DNA fragments were selected by colony hybridization using total human DNA as a probe. These clones were then used to search for cross-hybridizing sequences as described above for TB1, and the cross-hybridizing clones were subsequently used to screen cDNA libraries. One of the cDNA clones discovered in this way (FH38) contained a long ORF (2496 bp), 78 bp of which were identical to the above-noted sequences in YS11. The ends of the FH38 cDNA clone were then used to initiate cDNA walking to extend the sequence. Eventually, 85 cDNA clones were isolated from normal colon, brain and liver cDNA libraries and found to encompass 8973 nucleotides of contiguous transcript. The gene corresponding to this transcript was named APC. When used as probes for Northern blot analysis, APC cDNA clones hybridized to a single transcript of approximately 9.5 kb, suggesting that the great majority of the gene product was represented in the cDNA clones obtained. Sequences from the 5' end of the APC gene were found in YAC 37HG4 but not in YAC 14FH1. However, the 3' end of the APC gene was found in 14FH1 as well as 37HG4. The yeast artificial chromosome of the present invention designated YAC 37HG4 has been deposited with the National Collection of Industrial and Marine Bacteria (NCIMB), P.O. Box 31, 135 Abbey Road, Aberdeen AB9 8DG, Scotland, prior to the filing of this patent application. The NCIMB Accession Number of YAC clone YAC 37HG4 is 40353. Analogously, the 5' end of the MCC coding region was found in YAC clones 19AA9 and 26GC3 but not 24ED6 or 14FH1, while the 3' end displayed the opposite pattern. Thus, MCC and APC transcription units pointed in opposite directions, with the direction of transcription going from centromeric to telomeric in the case of MCC, and telomeric to centromeric in the case of APC. PFGE analysis of YAC DNA digested with various restriction endonucleases showed that TB2 and SRP were between MCC and APC, and that the 3' ends of the coding

regions of MCC and APC were separated by approximately 150 kb (Figure 1B).

Sequence analysis of the APC cDNA clones revealed an open reading frame of 8,535 nucleotides. The 5' end of the ORF contained a methionine codon (codon 1) that was preceded by an in-frame stop codon 9 bp upstream, and the 3' end was followed by several in-frame stop codons. The protein produced by initiation at codon 1 would contain 2,842 amino acids (Figure 3). The results of database searching with the APC gene product were quite complex due to the presence of large segments with locally biased amino acid compositions. In spite of this, APC could be roughly divided into two domains. The N-terminal 25% of the protein had a high content of leucine residues (12%) and showed local sequence similarities to myosins, various intermediate filament proteins (e.g., desmin, vimentin, neurofilaments) and *Drosophila* armadillo/human plakoglobin. The latter protein is a component of adhesive junctions (desmosomes) joining epithelial cells (Franke et al., Proc. Natl. Acad. Sci. U.S.A., Vol. 86, p. 4027 (1989); Perfer et al., Cell, Vol. 63, p. 1167 (1990)). The C-terminal 75% of APC (residues 731-2832) is 17% serine by composition with serine residues more or less uniformly distributed. This large domain also contains local concentrations of charged (mostly acidic) and proline residues. There was no indication of potential signal peptides, transmembrane regions, or nuclear targeting signals in APC, suggesting a cytoplasmic localization.

To detect short similarities to APC, a database search was performed using the PAM-40 matrix (Altschul. J. Mol. Bio., Vol. 219, p. 555 (1991)). Potentially interesting matches to several proteins were found. The most suggestive of these involved the *ral2* gene product of yeast, which is implicated in the regulation of ras activity (Fukui et al., Mol. Cell. Biol., Vol. 9, p. 5617 (1989)). Little is known about how *ral2* might interact with ras but it is interesting to note the positively-charged character of this region in the context of the negatively-charged GAP interaction region of ras. A specific electrostatic interaction between ras and GAP-related proteins has been proposed.

Because of the proximity of the MCC and APC genes, and the fact that both are implicated in colorectal tumorigenesis, we searched for similarities between the two predicted proteins. Bourne has previously noted that MCC has the potential to form alpha helical coiled coils (Nature, Vol. 351, p. 188 (1991)). Lupas and colleagues have recently developed a program for predicting coiled coil potential from primary sequence data (Science, Vol. 252, p. 1162 (1991)) and we have used their program to analyze both MCC and APC. Analysis of MCC indicated a discontinuous pattern of coiled-coil domains separated by putative "hinge" or "spacer" regions similar to those seen in laminin and other intermediate filament proteins. Analysis of the APC sequence revealed two regions in the N-terminal domain which had strong coiled coil-forming potential, and these regions corresponded to those that showed local similarities with myosin and IF proteins on database searching. In addition, one other putative coiled coil region was identified in the central region of APC. The potential for both APC and MCC to form coiled coils is interesting in that such structures often mediate homo- and hetero-oligomerization.

Finally, it had previously been noted that MCC shared a short similarity with the region of the m3 muscarinic acetylcholine receptor (mAChR) known to regulate specificity of G-protein coupling. The APC gene also contained a local similarity to the region of the m3 mAChR that overlapped with the MCC similarity (Figure 4B). Although the similarities to ral2 (Figure 4A) and m3 mAChR (Figure 4B) were not statistically significant, they were intriguing in light of previous observations relating G-proteins to neoplasia.

Each of the six genes described above was expressed in normal colon mucosa, as indicated by their representation in colon cDNA libraries. To study expression of the genes in neoplastic colorectal epithelium, we employed reverse transcription-polymerase chain reaction (PCR) assays. Primers based on the sequences of FER, TB1, TB2, MCC, and APC were each used to design primers for PCR performed with cDNA templates. Each of these genes was found to be expressed in normal colon, in each of ten cell lines derived from colorectal cancers, and in tumor cell lines derived from lung and bladder tumors. The

ten colorectal cancer cell lines included eight from patients with sporadic CRC and two from patients with FAP.

### Example 2

This example demonstrates a genetic analysis of the role of the FER gene in FAP and sporadic colorectal cancers.

We considered FER as a candidate because of its proximity to the FAP locus as judged by physical and genetic criteria (see Example 1), and its homology to known tyrosine kinases with oncogenic potential. Primers were designed to PCR-amplify the complete coding sequence of FER from the RNA of two colorectal cancer cell lines derived from FAP patients. cDNA was generated from RNA and used as a template for PCR. The primers used were 5'-AGAAGGATCCCTTGTGCAGTGTGGA-3' and 5'-GACAGGATCCTGAAGCTGAGTTTG-3'. The underlined nucleotides were altered from the true FER sequence to create BamHI sites. The cell lines used were JW and Difi, both derived from colorectal cancers of FAP patients. (C. Paraskeva, B.G. Buckle, D. Sheer, C.B. Wigley, *Int. J. Cancer* 34, 49 (1984); M.E. Gross et al., *Cancer Res.* 51, 1452 (1991). The resultant 2554 basepair fragments were cloned and sequenced in their entirety. The PCR products were cloned in the BamHI site of Bluescript SK (Stratagene) and pools of at least 50 clones were sequenced en masse using T7 polymerase, as described in Nigro et al., *Nature* 342, 705 (1989).

Only a single conservative amino acid change (GTG->CTG, creating a val to leu substitution at codon 439) was observed. The region surrounding this codon was then amplified from the DNA of individuals without FAP and this substitution was found to be a common polymorphism, not specifically associated with FAP. Based on these results, we considered it unlikely (though still possible) the FER gene was responsible for FAP. To amplify the regions surrounding codon 439, the following primers were used: 5'-TCAGAAAGTGCTGAAGAG-3' and 5'-GGAATAATTAGGTCTCCAA-3'. PCR products were digested with PstI, which yields a 50 bp fragment if codon 439 is leucine, but 26 and 24 bp fragments if it is valine. The primers used for sequencing were chosen from the FER cDNA sequence in Hao et al., supra.

### Example 3

This example demonstrates the genetic analysis of MCC, TB2, SRP and APC in FAP and sporadic colorectal tumors. Each of these genes is linked and encompassed by contig 3 (see Figure 1).

Several lines of evidence suggested that this contig was of particular interest. First, at least three of the four genes in this contig were within the deleted region identified in two FAP patients. (See Example 5 infra.) Second, allelic deletions of chromosome 5q21 in sporadic cancers appeared to be centered in this region. (Ashton-Rickardt et al., *Oncogene*, in press; and Miki et al., *Japn. J. Cancer Res.*, in press.) Some tumors exhibited loss of proximal RFLP markers (up to and potentially including the 5' end of MCC), but no loss of markers distal to MCC. Other tumors exhibited loss of markers distal to and perhaps including the 3' end of MCC, but no loss of sequences proximal to MCC. This suggested either that different ends of MCC were affected by loss in all such cases, or alternatively, that two genes (one proximal to and perhaps including MCC, the other distal to MCC) were separate targets of deletion. Third, clones from each of the six FAP region genes were used as probes on Southern blots containing tumor DNA from patients with sporadic CRC. Only two examples of somatic changes were observed in over 200 tumors studied: a rearrangement/deletion whose centromeric end was located within the MCC gene (Kinzler et al., supra) and an 800 bp insertion within the APC gene between nucleotides 4424 and 5584. Fourth, point mutations of MCC were observed in two tumors (Kinzler et al.) supra strongly suggesting that MCC was a target of mutation in at least some sporadic colorectal cancers.

Based on these results, we attempted to search for subtle alterations of contig 3 genes in patients with FAP. We chose to examine MCC and APC, rather than TB2 or SRP, because of the somatic mutations in MCC and APC noted above. To facilitate the identification of subtle alterations, the genomic sequences of MCC and APC exons were determined (see Table I). These sequences were used to design primers for PCR analysis of constitutional DNA from FAP patients.

We first amplified eight exons and surrounding introns of the MCC gene in affected individuals from 90 different FAP kindreds. The PCR products were analyzed by a ribonuclease (RNase) protein assay. In brief, the PCR products were hybridized to in vitro transcribed RNA probes representing the normal genomic sequences. The hybrids were digested with RNase A, which can cleave at single base pair mismatches within DNA-RNA hybrids, and the cleavage products were visualized following denaturing gel electrophoresis. Two separate RNase protection analyses were performed for each exon, one with the sense and one with the antisense strand. Under these conditions, approximately 40% of all mismatches are detectable. Although some amino acid variants of MCC were observed in FAP patients, all such variants were found in a small percentage of normal individuals. These variants were thus unlikely to be responsible for the inheritance of FAP.

We next examined three exons of the APC gene. The three exons examined included those containing nt 822-930, 931-1309, and the first 300 nt of the most distal exon (nt 1956-2256). PCR and RNase protection analysis were performed as described in Kinzler et al. supra, using the primers underlined in Table I. The primers for nt 1956-2256 were 5'-GCAAATCCTAAGAGAGAAACA-3' and 5'-GATGGCAAGCTTGAGCCAG-3'.

In 90 kindreds, the RNase protection method was used to screen for mutations and in an additional 13 kindreds, the PCR products were cloned and sequenced to search for mutations not detectable by RNase protection. PCR products were cloned into a Bluescript vector modified as described in T.A. Holton and M.W. Graham, Nucleic Acids Res. 19, 1156 (1991). A minimum of 100 clones were pooled and sequenced. Five variants were detected among the 103 kindreds analyzed. Cloning and subsequent DNA sequencing of the PCR product of patient P21 indicated a C to T transition in codon 413 that resulted in a change from arginine to cysteine. This amino acid variant was not observed in any of 200 DNA samples from individuals without FAP. Cloning and sequencing of the PCR product from patients P24 and P34, who demonstrated the same abnormal RNase protection pattern indicated that

both had a C to T transition at codon 301 that resulted in a change from arginine (CGA) to a stop codon (TGA). This change was not present in 200 individuals without FAP. As this point mutation resulted in the predicted loss of the recognition site for the enzyme Taq I, appropriate PCR products could be digested with Taq I to detect the mutation. This allowed us to determine that the stop codon co-segregated with disease phenotype in members of the family of P24. The inheritance of this change in affected members of the pedigree provides additional evidence for the importance of the mutation.

Cloning and sequencing of the PCR product from FAP patient P93 indicated a C to G transversion at codon 279, also resulting in a stop codon (change from TCA to TGA). This mutation was not present in 200 individuals without FAP. Finally, one additional mutation resulting in a serine (TCA) to stop codon (TGA) at codon 712 was detected in a single patient with FAP (patient P60).

The five germline mutations identified are summarized in Table IIA, as well as four others discussed in Example 9. In addition to these germline mutations, we identified several somatic mutations of MCC and APC in sporadic CRC's. Seventeen MCC exons were examined in 90 sporadic colorectal cancers by RNase protection analysis. In each case where an abnormal RNase protection pattern was observed, the corresponding PCR products were cloned and sequenced. This led to the identification of six point mutations (two described previously) (Kinzler et al., supra), each of which was not found in the germline of these patients (Table IIB). Four of the mutations resulted in amino acid substitutions and two resulted in the alteration of splice site consensus elements. Mutations at analogous splice site positions in other genes have been shown to alter RNA processing in vivo and in vitro.

Three exons of APC were also evaluated in sporadic tumors. Sixty tumors were screened by RNase protection, and an additional 98 tumors were evaluated by sequencing. The exons examined included nt 822-930, 931-1309, and 1406-1545 (Table I). A total of three mutations were identified, each of which proved to be somatic. Tumor T27 contained a somatic mutation of CGA (arginine) to TGA (stop codon) at codon 33. Tumor T135 contained a GT to GC change at a splice donor



site. Tumor T34 contained a 5 bp insertion (CAGCC between codons 288 and 289) resulting in a stop at codon 291 due to a frameshift.

We serendipitously discovered one additional somatic mutation in a colorectal cancer. During our attempt to define the sequences and splice patterns of the MCC and APC gene products in colorectal epithelial cells, we cloned cDNA from the colorectal cancer cell line SW480. The amino acid sequence of the MCC gene from SW480 was identical to that previously found in clones from human brain. The sequence of APC in SW480 cells, however, differed significantly, in that a transition at codon 1338 resulted in a change from glutamine (CAG) to a stop codon (TAG). To determine if this mutation was somatic, we recovered DNA from archival paraffin blocks of the original surgical specimen (T201) from which the tumor cell line was derived 28 years ago.

DNA was purified from paraffin sections as described in S.E. Goelz, S.R. Hamilton, and B. Vogelstein. *Biochem. Biophys. Res. Comm.* 130, 118 (1985). PCR was performed as described in reference 24, using the primers 5'-GTTCCAGCAGTGTCACAG-3' and 5'-GGGAGATTTCGCTCCTGA-3'. A PCR product containing codon 1338 was amplified from the archival DNA and used to show that the stop codon represented a somatic mutation present in the original primary tumor and in cell lines derived from the primary and metastatic tumor sites, but not from normal tissue of the patient.

The ten point mutations in the MCC and APC genes so far discovered in sporadic CRCs are summarized in Table IIB. Analysis of the number of mutant and wild-type PCR clones obtained from each of these tumors showed that in eight of the ten cases, the wild-type sequence was present in approximately equal proportions to the mutant. This was confirmed by RFLP analysis using flanking markers from chromosome 5q which demonstrated that only two of the ten tumors (T135 and T201) exhibited an allelic deletion on chromosome 5q. These results are consistent with previous observations showing that 20-40% of sporadic colorectal tumors had allelic deletions of chromosome 5q. Moreover, these data suggest that mutations of 5q21 genes

are not limited to those colorectal tumors which contain allelic deletions of this chromosome.

Example 4

This example characterizes small, nested deletions in DNA from two unrelated FAP patients.

DNA from 40 FAP patients was screened with cosmids that had been mapped into a region near the APC locus to identify small deletions or rearrangements. Two of these cosmids, L5.71 and L5.79, hybridized with a 1200 kb NotI fragment in DNAs from most of the FAP patients screened.

The DNA of one FAP patient, 3214, showed only a 940 kb NotI fragment instead of the expected 1200 kb fragment. DNA was analyzed from four other members of the patient's immediate family; the 940 kb fragment was present in her affected mother (4711), but not in the other, unaffected family members. The mother also carried a normal 1200 kb NotI fragment that was transmitted to her two unaffected offspring. These observations indicated that the mutant polyposis allele is on the same chromosome as the 940 kb NotI fragment. A simple interpretation is that APC patients 3214 and 4711 each carry a 260 kb deletion within the APC locus.

If a deletion were present, then other enzymes might also be expected to produce fragments with altered mobilities. Hybridization of L5.79 to NruI-digested DNAs from both affected members of the family revealed a novel NruI fragment of 1300 kb, in addition to the normal 1200 kb NruI fragment. Furthermore, MluI fragments in patients 3214 and 4711 also showed an increase in size consistent with the deletion of an MluI site. The two chromosome 5 homologs of patient 3214 were segregated in somatic cell hybrid lines; HHW1155 (deletion hybrid) carried the abnormal homolog and HHW1159 (normal hybrid) carried the normal homolog.

Because patient 3214 showed only a 940 kb NotI fragment, she had not inherited the 1200 kb fragment present in the unaffected father's DNA. This observation suggests that he must be heterozygous for, and have transmitted, either a deletion of the L5.79 probe region or a variant NotI fragment too large to resolve on the gel system. As

expected, the hybrid cell line HHW1159, which carries the paternal homolog, revealed no resolved Not fragment when probed with L5.79. However, probing of HHW1159 DNA with L5.79 following digestion with other enzymes did reveal restriction fragments, demonstrating the presence of DNA homologous to the probe. The father is, therefore, interpreted as heterozygous for a polymorphism at the NotI site, with one chromosome 5 having a 1200 kb NotI fragment and the other having a fragment too large to resolve consistently on the gel. The latter was transmitted to patient 3214.

When double digests were used to order restriction sites within the 1200 kb NotI fragment, L5.71 and L5.79 were both found to lie on a 550 kb NotI-NruI fragment and, therefore, on the same side of an NruI site in the 1200 kb NotI fragment. To obtain genomic representation of sequences present over the entire 1200 kb NotI fragment, we constructed a library of small-fragment inserts enriched for sequences from this fragment. DNA from the somatic cell hybrid HHW141, which contains about 40% of chromosome 5, was digested with NotI and electrophoresed under pulsed-field gel (PFG) conditions; EcoRI fragments from the 1200 kb region of this gel were cloned into a phage vector. Probe Map30 was isolated from this library. In normal individuals probe Map30 hybridizes to the 1200 kb NotI fragment and to a 200 kb NruI fragment. This latter hybridization places Map30 distal, with respect to the locations of L5.71 and L5.79, to the NruI site of the 550 kb NotI-NruI fragment.

Because Map30 hybridized to the abnormal, 1300 kb NruI fragment of patient 3214, the locus defined by Map30 lies outside the hypothesized deletion. Furthermore, in normal chromosomes Map30 identified a 200 kb NruI fragment and L5.79 identified a 1200 kb NruI fragment; the hypothesized deletion must, therefore, be removing an NruI site, or sites, lying between Map30 and L5.79, and these two probes must flank the hypothesized deletion. A restriction map of the genomic region, showing placement of these probes, is shown in Figure 5.

A NotI digest of DNA from another FAP patient, 3824, was probed with L5.79. In addition to the 1200 kb normal NotI fragment, a

fragment of approximately 1100 kb was observed, consistent with the presence of a 100 kb deletion in one chromosome 5. In this case, however, digestion with *Nru*I and *Mlu*I did not reveal abnormal bands, indicating that if a deletion were present, its boundaries must lie distal to the *Nru*I and *Mlu*I sites of the fragments identified by L5.79. Consistent with this expectation, hybridization of Map30 to DNA from patient 3824 identified a 760 kb *Mlu*I fragment in addition to the expected 860 kb fragment, supporting the interpretation of a 100 kb deletion in this patient. The two chromosome 5 homologs of patient 3824 were segregated in somatic cell hybrid lines; HHW1291 was found to carry only the abnormal homolog and HHW1290 only the normal homolog.

That the 860 kb *Mlu*I fragment identified by Map30 is distinct from the 830 kb *Mlu*I fragment identified previously by L5.79 was demonstrated by hybridization of Map30 and L5.79 to a *Not*I-*Mlu*I double digest of DNA from the hybrid cell (HHW1159) containing the nondeleted chromosome 5 homolog of patient 3214. As previously indicated, this hybrid is interpreted as missing one of the *Not*I sites that define the 1200 kb fragment. A 620 kb *Not*I-*Mlu*I fragment was seen with probe L5.79, and an 860 kb fragment was seen with Map30. Therefore, the 830 kb *Mlu*I fragment recognized by probe L5.79 must contain a *Not*I site in HHW1159 DNA; because the 860 kb *Mlu*I fragment remains intact, it does not carry this *Not*I site and must be distinct from the 830 kb *Mlu*I fragment.

#### Example 5

This example demonstrates the isolation of human sequences which span the region deleted in the two unrelated FAP patients characterized in Example 4.

A strong prediction of the hypothesis that patients 3214 and 3824 carry deletions is that some sequences present on normal chromosome 5 homologs would be missing from the hypothesized deletion homologs. Therefore, to develop genomic probes that might confirm the deletions, as well as to identify genes from the region, YAC clones from a contig seeded by cosmid L5.79 were localized from a library containing seven haploid human genome equivalents (Albertsen et al.,

Proc. Natl. Acad. Sci. U.S.A., Vol. 87, pp. 4256-4260 (1990)) with respect to the hypothesized deletions. Three clones, YACs 57B8, 310D8, and 183H12, were found to overlap the deleted region.

Importantly, one end of YAC 57B8 (clone AT57) was found to lie within the patient 3214 deletion. Inverse polymerase chain reaction (PCR) defined the end sequences of the insert of YAC 57B8. PCR primers based on one of these end sequences repeatedly failed to amplify DNA from the somatic cell hybrid (HHW1155) carrying the deleted homolog of patient 3214, but did amplify a product of the expected size from the somatic cell hybrid (HHW1159) carrying the normal chromosome 5 homolog. This result supported the interpretation that the abnormal restriction fragments found in the DNA of patient 3214 result from a deletion.

Additional support for the hypothesis of deletion in DNA from patient 3214 came from subcloned fragments of YAC 183H12, which spans the region in question. Y11, an EcoRI fragment cloned from YAC 183H12, hybridized to the normal, 1200 kb NotI fragment of patient 4711, but failed to hybridize to the abnormal, 940 kb NotI fragment of 4711 or to DNA from deletion cell line HHW1155. This result confirmed the deletion in patient 3214.

Two additional EcoRI fragments from YAC 183H12, Y10 and Y14, were localized within the patient 3214 deletion by their failure to hybridize to DNA from HHW1155. Probe Y10 hybridizes to a 150 kb NruI fragment in normal chromosome 5 homologs. Because the 3214 deletion creates the 1300 kb NruI fragment seen with the probes L5.79 and Map30 that flank the deletion, these NruI sites and the 150 kb NruI fragment lying between must be deleted in patient 3214. Furthermore, probe Y10 hybridizes to the same 620 kb NotI-MluI fragment seen with probe L5.79 in normal DNA, indicating its location as L5.79-proximal to the deleted MluI site and placing it between the MluI site and the L5.79-proximal NruI site. The MluI site must, therefore, lie between the NruI sites that define the 150 kb NruI fragment (see Figure 5).

Probe Y11 also hybridized to the 150 kb NruI fragment in the normal chromosome 5 homolog, but failed to hybridize to the 620 kb NotI-MluI fragment, placing it L5.79-distal to the MluI site, but

proximal to the second NruI site. Hybridization to the same (860 kb) MluI fragment as Map30 confirmed the localization of probe Y11 L5.79-distal to the MluI site.

Probe Y14 was shown to be L5.79-distal to both deleted NruI sites by virtue of its hybridization to the same 200 kb NruI fragment of the normal chromosome 5 seen with Map30. Therefore, the order of these EcoRI fragments derived from YAC 183H12 and deleted in patient 3214, with respect to L5.79 and Map30, is L5.79-Y10-Y11-Y14-Map30.

The 100 kb deletion of patient 3824 was confirmed by the failure of aberrant restriction fragments in this DNA to hybridize with probe Y11, combined with positive hybridizations to probes Y10 and/or Y14. Y10 and Y14 each hybridized to the 1100 kb NotI fragment of patient 3824 as well as to the normal 1200 kb NotI fragment, but Y11 hybridized to the 1200 kb fragment only. In the MluI digest, probe Y14 hybridized to the 860 kb and 760 kb fragments of patient 3824 DNA, but probe Y11 hybridized only to the 860 kb fragment. We conclude that the basis for the alteration in fragment size in DNA from patient 3824 is, indeed, a deletion. Furthermore, because probes Y10 and Y14 are missing from the deleted 3214 chromosome, but present on the deleted 3824 chromosome, and they have been shown to flank probe Y11, the deletion in patient 3824 must be nested within the patient 3214 deletion.

Probes Y10, Y11, Y14 and Map30 each hybridized to YAC 310D8, indicating that this YAC spanned the patient 3824 deletion and at a minimum, most of the 3214 deletion. The YAC characterizations, therefore, confirmed the presence of deletions in the patients and provided physical representation of the deleted region.

#### Example 6

This example demonstrates that the MCC coding sequence maps outside of the region deleted in the two FAP patients characterized in Example 4.

An intriguing FAP candidate gene, MCC, recently was ascertained with cosmid L5.71 and was shown to have undergone mutation in colon carcinomas (Kinzler et al., supra). It was therefore of interest to

map this gene with respect to the deletions in APC patients. Hybridization of MCC probes with an overlapping series of YAC clones extending in either direction from L5.71 showed that the 3' end of MCC must be oriented toward the region of the two APC deletions.

Therefore, two 3' cDNA clones from MCC were mapped with respect to the deletions: clone 1CI (bp 2378-4181) and clone 7 (bp 2890-3560). Clone 1CI contains sequences from the C-terminal end of the open reading frame, which stops at nucleotide 2708, as well as 3' untranslated sequence. Clone 7 contains sequence that is entirely 3' to the open reading frame. Importantly, the entire 3' untranslated sequence contained in the cDNA clones consists of a single 2.5 kb exon. These two clones were hybridized to DNAs from the YACs spanning the FAP region. Clone 7 fails to hybridize to YAC 310D8, although it does hybridize to YACs 183H12 and 57B8; the same result was obtained with the cDNA 1CI. Furthermore, these probes did show hybridization to DNAs from both hybrid cell lines (HWW1159 and HWW1155) and the lymphoblastoid cell line from patient 3214, confirming their locations outside the deleted region. Additional mapping experiments suggested that the 3' end of the MCC cDNA clone contig is likely to be located more than 45 kb from the deletion of patient 3214 and, therefore, more than 100 kb from the deletion of patient 3824.

#### Example 7

This example identifies three genes within the deleted region of chromosome 5 in the two unrelated FAP patients characterized in Example 4.

Genomic clones were used to screen cDNA libraries in three separate experiments. One screening was done with a phage clone derived from YAC 310D8 known to span the 260 kb deletion of patient 3214. A large-insert phage library was constructed from this YAC; screening with Y11 identified  $\lambda$ 205, which mapped within both deletions. When clone  $\lambda$ 205 was used to probe a random-, plus oligo(dT)-, primed fetal brain cDNA library (approximately 300,000 phage), six cDNA clones were isolated and each of them mapped entirely within both deletions. Sequence analysis of these six clones formed a single cDNA contig, but did not reveal an extended open reading frame. One

of the six cDNAs was used to isolate more cDNA clones, some of which crossed the L5.71-proximal breakpoint of the 3824 deletion, as indicated by hybridization to both chromosome of this patient. These clones also contained an open reading frame, indicating a transcriptional orientation proximal to distal with respect to L5.71. This gene was named DP1 (deleted in polyposis 1). This gene is identical to TB2 described above.

cDNA walks yielded a cDNA contig of 3.0-3.5 kb, and included two clones containing terminal poly(A) sequences. This size corresponds to the 3.5 kb band seen by Northern analysis. Sequencing of the first 3163 bp of the cDNA contig revealed an open reading frame extending from the first base to nucleotide 631, followed by a 2.5 kb 3' untranslated region. The sequence surrounding the methionine codon at base 77 conforms to the Kozak consensus of an initiation methionine (Kozak, 1984). Failed attempts to walk farther, coupled with the similarity of the lengths of isolated cDNA and mRNA, suggested that the NH<sub>2</sub>-terminus of the DP1 protein had been reached. Hybridization to a combination of genomic and YAC DNAs cut with various enzymes indicated the genomic coverage of DP1 to be approximately 30 kb.

Two additional probes for the locus, YS-11 and YS-39, which had been ascertained by screening of a cDNA library with an independent YAC probe identified with MCC sequences adjacent to L5.71, were mapped into the deletion region. YS-39 was shown to be a cDNA identical in sequence to DP1. Partial characterization of YS-11 had shown that 200 bp of DNA sequence at one end was identical to sequence coding for the 19 kd protein of the ribosomal signal recognition particle, SRP19 (Lingelbach et al., supra). Hybridization experiments mapped YS-11 within both deletions. The sequence of this clone, however, was found to be complex. Although 454 bp of the 1032 bp sequence of YS-11 were identical to the GenBank entry for the SRP19 gene, another 578 bp appended 5' to the SRP19 sequence was found to consist of previously unreported sequence containing no extended open reading frames. This suggested that YS-11 was either a chimeric clone containing two independent inserts or a clone of an incompletely processed or aberrant message. If YS-11 were a conventional chimeric clone, the



independent segments would not be expected to map to the same physical region. The segments resulting from anomalous processing of a continuous transcript, however, would map to a single chromosomal region.

Inverse PCR with primers specific to the two ends of YS-11, the SRP19 end and the unidentified region, verified that both sequences map within the YAC 310D8; therefore, YS-11 is most likely a clone of an immature or anomalous mRNA species. Subsequently, both ends were shown to lie with the deleted region of patient 3824, and YS-11 was used to screen for additional cDNA clones.

Of the 14 cDNA clones selected from the fetal brain library, one clone, V5, was of particular interest in that it contained an open reading frame throughout, although it included only a short identity to the first 78 5' bases of the YS-11 sequence. Following the 78 bp of identical sequence, the two cDNA sequences diverged at an AG. Furthermore, divergence from genomic sequence was also seen after these 78 bp, suggesting the presence of a splice junction, and supporting the view that YS-11 represents an irregular message.

Starting with V5, successive 5' and 3' walks were performed; the resulting cDNA contig consisted of more than 100 clones, which defined a new transcript, DP2. Clones walking in the 5' direction crossed the 3824 deletion breakpoint farthest from L5.71; since its 3' end is closer to this cosmid than its 5' end, the transcriptional orientation of DP2 is opposite to that of MCC and DP1.

The third screening approach relied on hybridization with a 120 kb MluI fragment from YAC 57B8. This fragment hybridizes with probe Y11 and completely spans the 100 kb deletion in patient 3824. the fragment was purified on two preparative PFGs, labeled, and used to screen a fetal brain cDNA library. A number of cDNA clones previously identified in the development of the DP1 and DP2 contigs were reascertained. However, 19 new cDNA clones mapped into the patient 3824 deletion. Analysis indicated that these 19 formed a new contig, DP3, containing a large open reading frame.

A clone from the 5' end of this new cDNA contig hybridized to the same EcoRI fragment as the 3' end of DP2. Subsequently, the DP2

and DP3 contigs were connected by a single 5' walking step from DP3, to form the single contig DP2.5. The complete nucleotide sequence of DP2.5 is shown in Figure 9.

The consensus cDNA sequence of DP2.5 suggests that the entire coding sequence of DP2.5 has been obtained and is 8532 bp long. The most 5' ATG codon occurs two codons from an in-frame stop and conforms to the Kozak initiation consensus (Kozak, Nucl. Acids. Res., Vol. 12, p. 857-872, 1984). The 3' open reading frame breaks down over the final 1.8 kb, giving multiple stops in all frames. A poly(A) sequence was found in one clone approximately 1 kb into the 3' untranslated region, associated with a polyadenylation signal 33 bp upstream (position 9530). The open reading frame is almost identical to that identified as APC above.

An alternatively spliced exon at nucleotide 934 of the DP2.5 transcript is of potential interest. It was first discovered by noting that two classes of cDNA had been isolated. The more abundant cDNA class contains a 303 bp exon not included in the other. The presence in vivo of the two transcripts was verified by an exon connection experiment. Primers flanking the alternatively spliced exon were used to amplify, by PCR, cDNA prepared from various adult tissues. Two PCR products that differed in size by approximately 300 bases were amplified from all the tissues tested; the larger product was always more abundant than the smaller.

#### Example 8

This example demonstrates the primers used to identify subtle mutations in DP1, SRP19, and DP25.

To obtain DNA sequence adjacent to the exons of the genes DP1, DP2.5, and SRP19, sequencing substrate was obtained by inverse PCR amplification of DNAs from two YACs, 310D8 and 183H12, that span the deletions. Ligation at low concentration cyclized the restriction enzyme-digested YAC DNAs. Oligonucleotides with sequencing tails, designed in inverse orientation at intervals along the cDNAs, primed PCR amplification from the cyclized templates. Comparison of these DNA sequences with the cDNA sequences placed exon boundaries at the divergence points. SRP19 and DP1 were each shown to have five

exons. DP2.5 consisted of 15 exons. The sequences of the oligonucleotides synthesized to provide PCR amplification primers for the exons of each of these genes are listed in Table III. With the exception of exons 1, 3, 4, 9, and 15 of DP2.5 (see below), the primer sequences were located in intron sequences flanking the exons. The 5' primer of exon 1 is complementary to the cDNA sequence, but extends just into the 5' Kozak consensus sequence for the initiator methionine, allowing a survey of the translated sequences. The 5' primer of exon 3 is actually in the 5' coding sequences of this exon, as three separate intronic primers simply would not amplify. The 5' primer of exon 4 just overlaps the 5' end of this exon, and we thus fail to survey the 19 most 5' bases of this exon. For exon 9, two overlapping primer sets were used, such that each had one end within the exon. For exon 15, the large 3' exon of DP2.5, overlapping primer pairs were placed along the length of the exon; each pair amplified a product of 250-400 bases.

#### Example 9

This example demonstrates the use of single stranded conformation polymorphism (SSCP) analysis as described by Orita et al. Proc. Natl. Acad. Sci. U.S.A., Vol. 86, pp. 2766-70 (1989) and Genomics, Vol. 5, pp. 874-879 (1989) as applied to DP1, SRP19 and DP2.5.

SSCP analysis identifies most single- or multiple-base changes in DNA fragments up to 400 bases in length. Sequence alterations are detected as shifts in electrophoretic mobility of single-stranded DNA on nondenaturing acrylamide gels; the two complementary strands of a DNA segment usually resolve as two SSCP conformers of distinct mobilities. However, if the sample is from an individual heterozygous for a base-pair variant within the amplified segment, often three or more bands are seen. In some cases, even the sample from a homozygous individual will show multiple bands. Base-pair-change variants are identified by differences in pattern among the DNAs of the sample set.

Exons of the candidate genes were amplified by PCR from the DNAs of 61 unrelated FAP patients and a control set of 12 normal individuals. The five exons from DP1 revealed no unique conformers in the FAP patients, although common conformers were observed with exons

2 and 3 in some individuals of both affected and control sets, indicating the presence of DNA sequence polymorphisms. Likewise, none of the five exons of SRP19 revealed unique conformers in DNA from FAP patients in the test panel.

Testing of exons 1 through 14 and primer sets A through N of exon 15 of the DP2.5 gene, however, revealed variant conformers specific to FAP patients in exons 7, 8, 10, 11, and 15. These variants were in the unrelated patients 3746, 3460, 3827, 3712, and 3751, respectively. The PCR-SSCP procedure was repeated for each of these exons in the five affected individuals and in an expanded set of 48 normal controls. The variant bands were reproducible in the FAP patients but were not observed in any of the control DNA samples. Additional variant conformers in exons 11 and 15 of the DP2.5 gene were seen; however, each of these was found in both the affected and control DNA sets. The five sets of conformers unique to the FAP patients were sequenced to determine the nucleotide changes responsible for their altered mobilities. The normal conformers from the host individuals were sequenced also. Bands were cut from the dried acrylamide gels, and the DNA was eluted. PCR amplification of these DNAs provided template for sequencing.

The sequences of the unique conformers from exons 7, 8, 10, and 11 of DP2.5 revealed dramatic mutations in the DP2.5 gene. The sequence of the new mutation creating the exon 7 conformer in patient 3746 was shown to contain a deletion of two adjacent nucleotides, at positions 730 and 731 in the cDNA sequence (Figure 7). The normal sequence at this splice junction is CAGGGTCA (intronic sequence underlined), with the intron-exon boundary between the two repetitions of AG. The mutant allele in this patient has the sequence CAGGTCA. Although this change is at the 5' splice site, comparison with known consensus sequences of splice junctions would suggest that a functional splice junction is maintained. If this new splice junction were functional, the mutation would introduce a frameshift that creates a stop codon 15 nucleotides downstream. If the new splice junction were not functional, messenger processing would be significantly altered.

To confirm the 2-base deletion, the PCR product from FAP patient 3746 and a control DNA were electrophoresed on an acrylamide-urea denaturing gel, along with the products of a sequencing reaction. The sample from patient 3746 showed two bands differing in size by 2 nucleotides, with the larger band identical in mobility to the control sample; this result was independent confirmation that patient 3746 is heterozygous for a 2 bp deletion.

The unique conformer found in exon 8 of patient 3460 was found to carry a C-T transition, at position 904 in the cDNA sequence of DP2.5 (shown in Figure 7), which replaced the normal sequence of CGA with TGA. This point mutation, when read in frame, results in a stop codon replacing the normal arginine codon. This single-base change had occurred within the context of a CG dimer, a potential hot spot for mutation (Barker et al., 1984).

The conformer unique to FAP patient 3827 in exon 10 was found to contain a deletion of one nucleotide (1367, 1368, or 1369) when compared to the normal sequence found in the other bands on the SSCP gel. This deletion, occurring within a set of three T's, changed the sequence from CTTTCA to CTTCA; this 1 base frameshift creates a downstream stop within 30 bases. The PCR product amplified from this patient's DNA also was electrophoresed on an acrylamide-urea denaturing gel, along with the PCR product from a control DNA and products from a sequencing reaction. The patient's PCR product showed two bands differing by 1 bp in length, with the larger identical in mobility to the PCR product from the normal DNA; this result confirmed the presence of a 1 bp deletion in patient 3827.

Sequence analysis of the variant conformer of exon 11 from patient 3712 revealed the substitution of a T by a G at position 1500, changing the normal tyrosine codon to a stop codon.

The pair of conformers observed in exon 15 of the DP2.5 gene for FAP patient 3751 also was sequenced. These conformers were found to carry a nucleotide substitution of C to G at position 5253, the third base of a valine codon. No amino acid change resulted from this substitution, suggesting that this conformer reflects a genetically silent polymorphism.

The observation of distinct inactivating mutations in the DP2.5 gene in four unrelated patients strongly suggested that DP2.5 is the gene involved in FAP. These mutations are summarized in Table IIA.

Example 10

This example demonstrates that the mutations identified in the DP2.5 (APC) gene segregate with the FAP phenotype.

Patient 3746, described above as carrying an APC allele with a frameshift mutation, is an affected offspring of two normal parents. Colonoscopy revealed no polyps in either parent nor among the patient's three siblings.

DNA samples from both parents, from the patient's wife, and from their three children were examined. SSCP analysis of DNA from both of the patient's parents displayed the normal pattern of conformers for exon 7, as did DNA from the patient's wife and one of his offspring. The two other children, however, displayed the same new conformers as their affected father. Testing of the patient and his parents with highly polymorphic VNTR (variable number of tandem repeat) markers showed a 99.98% likelihood that they are his biological parents.

These observations confirmed that this novel conformer, known to reflect a 2 bp deletion mutation in the DP2.5 gene, appeared spontaneously with FAP in this pedigree and was transmitted to two of the children of the affected individual.

Example 11

This example demonstrates polymorphisms in the APC gene which appear to be unrelated to disease (FAP).

Sequencing of variant conformers found among controls as well as individuals with APC has revealed the following polymorphisms in the APC gene: first, in exon 11, at position 1458, a substitution of T to C creating an RsaI restriction site but no amino acid change; and second, in exon 15, at positions 5037 and 5271, substitutions of A to G and G to T, respectively, neither resulting in amino acid substitutions. These nucleotide polymorphisms in the APC gene sequence may be useful for diagnostic purposes.

**Example 12**

This example shows the structure of the APC gene.

The structure of the APC gene is schematically shown in Figure 8, with flanking intron sequences indicated.

The continuity of the very large (6.5 kb), most 3' exon in DP2.5 was shown in two ways. First, inverse PCR with primers spanning the entire length of this exon revealed no divergence of the cDNA sequence from the genomic sequence. Second, PCR amplification with converging primers placed at intervals along the exon generated products of the same size whether amplified from the originally isolated cDNA, cDNA from various tissues, or genomic template. Two forms of exon 9 were found in DP2.5: one is the complete exon; and the other, labeled exon 9A, is the result of a splice into the interior of the exon that deletes bases 934 to 1236 in the mRNA and removes 101 amino acids from the predicted protein (see Figure 7).

**Example 13**

This example demonstrates the mapping of the FAP deletions with respect to the APC exons.

Somatic cell hybrids carrying the segregated chromosomes 5 from the 100 kb (HHW1291) and 260 kb (HHW1155) deletion patients were used to determine the distribution of the APC genes exons across the deletions. DNAs from these cell lines were used as template, along with genomic DNA from a normal control, for PCR-based amplification of the APC exons.

PCR analysis of the hybrids from the 260 kb deletion of patient 3214 showed that all but one (exon 1) of the APC exons are removed by this deletion. PCR analysis of the somatic cell hybrid HHW1291, carrying the chromosome 5 homolog with the 100 kb deletion from patient 3824, revealed that exons 1 through 9 are present but exons 10 through 15 are missing. This result placed the deletion breakpoint either between exons 9 and 10 or within exon 10.

**Example 14**

This example demonstrates the expression of alternately spliced APC messenger in normal tissues and in cancer cell lines.

Tissues that express the APC gene were identified by PCR amplification of cDNA made to mRNA with primers located within adjacent APC exons. In addition, PCR primers that flank the alternatively spliced exon 9 were chosen so that the expression pattern of both splice forms could be assessed. All tissue types tested (brain, lung, aorta, spleen, heart, kidney, liver, stomach, placenta, and colonic mucosa) and cultured cell lines (lymphoblasts, HL60, and choriocarcinoma) expressed both splice forms of the APC gene. We note, however, that expression by lymphocytes normally residing in some tissues, including colon, prevents unequivocal assessment of expression. The large mRNA, containing the complete exon 9 rather than only exon 9A, appears to be the more abundant message.

Northern analysis of poly(A)-selected RNA from lymphoblasts revealed a single band of approximately 10 kb, consistent with the size of the sequenced cDNA.

#### Example 15

This example discusses structural features of the APC protein predicted from the sequence.

The cDNA consensus sequence of APC predicts that the longer, more abundant form of the message codes for a 2842 or 28444 amino acid peptide with a mass of 311.8 kd. This predicted APC peptide was compared with the current data bases of protein and DNA sequences using both Intelligenetics and GCG software packages. No genes with a high degree of amino acid sequence similarity were found. Although many short (approximately 20 amino acid) regions of sequence similarity were uncovered, none was sufficiently strong to reveal which, if any, might represent functional homology. Interestingly, multiple similarities to myosins and keratins did appear. The APC gene also was scanned for sequence motifs of known function; although multiple glycosylation, phosphorylation, and myristoylation sites were seen, their significance is uncertain.

Analysis of the APC peptide sequence did identify features important in considering potential protein structure. Hydropathy plots (Kyte and Doolittle, J. Mol. Biol. Vol. 157, pp. 105-132 (1982)) indicate that the APC protein is notably hydrophilic. No hydrophobic domains



suggesting a signal peptide or a membrane-spanning domain were found. Analysis of the first 1000 residues indicates that  $\alpha$ -helical rods may form (Cohen and Parry, Trends Biochem, Sci. Vol. 77, pp. 245-248 (1986); there is a scarcity of proline residues and, there are a number of regions containing heptad repeats (apolar-X-X-apolar-X-X-X). Interestingly, in exon 9A, the deleted form of exon 9, two heptad repeat regions are reconnected in the proper heptad repeat frame, deleting the intervening peptide region. After the first 1000 residues, the high proline content of the remainder of the peptide suggests a compact rather than a rod-like structure.

The most prominent feature of the second 1000 residues is a 20 amino acid repeat that is iterated seven times with semiregular spacing (Table 4). The intervening sequences between the seven repeat regions contained 114, 116, 151, 205, 107, and 58 amino acids, respectively. Finally, residues 2200-24000 contain a 200 amino acid basic domain.

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## (i) SEQUENCE CHARACTERISTICS:

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(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

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(vii) IMMEDIATE SOURCE:  
(B) CLONE: DP2.5 (APC)

(ix) FEATURE:  
(A) NAME/KEY: CDS  
(B) LOCATION: 34..8562

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGACTCGGAA ATGAGGTCCA AGGCTAGCCA AGG ATG GCT GCA GCT TCA TAT GAT	54
Met Ala Ala Ala Ser Tyr Asp	
1 5	
CAG TTG TTA AAG CAA GTT GAG GCA CTG AAG ATG GAG AAC TCA AAT CTT	102
Gln Leu Leu Lys Gln Val Glu Ala Leu Lys Met Glu Asn Ser Asn Leu	
10 15 20	
CGA CAA GAG CTA GAA GAT AAT TCC AAT CAT CTT ACA AAA CTG GAA ACT	150
Arg Gln Glu Leu Glu Asp Asn Ser Asn His Leu Thr Lys Leu Glu Thr	
25 30 35	
GAG GCA TCT AAT ATG AAG GAA GTA CTT AAA CAA CTA CAA GGA AGT ATT	198
Glu Ala Ser Asn Met Lys Glu Val Leu Lys Gln Leu Gln Gly Ser Ile	
40 45 50 55	
GAA GAT GAA GCT ATG GCT TCT TCT GGA CAG ATT GAT TTA TTA GAG CGT	246
Glu Asp Glu Ala Met Ala Ser Ser Gly Gln Ile Asp Leu Leu Glu Arg	
60 65 70	
CTT AAA GAG CTT AAC TTA GAT AGC AGT AAT TTC CCT GGA GTA AAA CTG	294
Leu Lys Glu Leu Asn Leu Asp Ser Ser Asn Phe Pro Gly Val Lys Leu	
75 80 85	
CGG TCA AAA ATG TCC CTC CGT TCT TAT GGA AGC CGG GAA GGA TCT GTA	342
Arg Ser Lys Met Ser Leu Arg Ser Tyr Gly Ser Arg Glu Gly Ser Val	
90 95 100	
TCA AGC CGT TCT GGA GAG TGC AGT CCT GTT CCT ATG GGT TCA TTT CCA	390
Ser Ser Arg Ser Gly Glu Cys Ser Pro Val Pro Met Gly Ser Phe Pro	
105 110 115	
AGA AGA GGG TTT GTA AAT GGA AGC AGA GAA AGT ACT GGA TAT TTA GAA	438
Arg Arg Gly Phe Val Asn Gly Ser Arg Glu Ser Thr Gly Tyr Leu Glu	
120 125 130 135	
GAA CTT GAG AAA GAG AGG TCA TTG CTT CTT GCT GAT CTT GAC AAA GAA	486
Glu Leu Glu Lys Glu Arg Ser Leu Leu Leu Ala Asp Leu Asp Lys Glu	
140 145 150	
GAA AAG GAA AAA GAC TGG TAT TAC GCT CAA CTT CAG AAT CTC ACT AAA	534
Glu Lys Glu Lys Asp Trp Tyr Tyr Ala Gln Leu Gln Asn Leu Thr Lys	
155 160 165	
AGA ATA GAT AGT CTT CCT TTA ACT GAA AAT TTT TCC TTA CAA ACA GAT	582
Arg Ile Asp Ser Leu Pro Leu Thr Glu Asn Phe Ser Leu Gln Thr Asp	
170 175 180	
TTG ACC AGA AGG CAA TTG GAA TAT GAA GCA AGG CAA ATC AGA GTT GCG	630
Leu Thr Arg Arg Gln Leu Glu Tyr Glu Ala Arg Gln Ile Arg Val Ala	
185 190 195	

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ATG GAA GAA CAA CTA GGT ACC TGC CAG GAT ATG GAA AAA CGA GCA CAG Met Glu Glu Gln Leu Gly Thr Cys Gln Asp Met Glu Lys Arg Ala Gln 200 205 210 215	678
CGA AGA ATA GCC AGA ATT CAG CAA ATC GAA AAG GAC ATA CTT CGT ATA Arg Arg Ile Ala Arg Ile Gln Gln Ile Glu Lys Asp Ile Leu Arg Ile 220 225 230	726
CGA CAG CTT TTA CAG TCC CAA GCA ACA GAA GCA GAG AGG TCA TCT CAG Arg Gln Leu Leu Gln Ser Gln Ala Thr Glu Ala Glu Arg Ser Ser Gln 235 240 245	774
AAC AAG CAT GAA ACC GGC TCA CAT GAT GCT GAG CGG CAG AAT GAA GGT Asn Lys His Glu Thr Gly Ser His Asp Ala Glu Arg Gln Asn Glu Gly 250 255 260	822
CAA GGA GTG GGA GAA ATC AAC ATG GCA ACT TCT GGT AAT GGT CAG GGT Gln Gly Val Gly Glu Ile Asn Met Ala Thr Ser Gly Asn Gly Gln Gly 265 270 275	870
TCA ACT ACA CGA ATG GAC CAT GAA ACA GCC AGT GTT TTG AGT TCT AGT Ser Thr Thr Arg Met Asp His Glu Thr Ala Ser Val Leu Ser Ser Ser 280 285 290 295	918
AGC ACA CAC TCT GCA CCT CGA AGG CTG ACA AGT CAT CTG GGA ACC AAG Ser Thr His Ser Ala Pro Arg Arg Leu Thr Ser His Leu Gly Thr Lys 300 305 310	966
GTG GAA ATG GTG TAT TCA TTG TTG TCA ATG CTT GGT ACT CAT GAT AAG Val Glu Met Val Tyr Ser Leu Leu Ser Met Leu Gly Thr His Asp Lys 315 320 325	1014
GAT GAT ATG TCG CGA ACT TTG CTA GCT ATG TCT AGC TCC CAA GAC AGC Asp Asp Met Ser Arg Thr Leu Leu Ala Met Ser Ser Ser Gln Asp Ser 330 335 340	1062
TGT ATA TCC ATG CGA CAG TCT GGA TGT CTT CCT CTC CTC ATC CAG CTT Cys Ile Ser Met Arg Gln Ser Gly Cys Leu Pro Leu Leu Ile Gln Leu 345 350 355	1110
TTA CAT GGC AAT GAC AAA GAC TCT GTA TTG TTG GGA AAT TCC CGG GGC Leu His Gly Asn Asp Lys Asp Ser Val Leu Leu Gly Asn Ser Arg Gly 360 365 370 375	1158
AGT AAA GAG GCT CGG GCC AGG GCC AGT GCA GCA CTC CAC AAC ATC ATT Ser Lys Glu Ala Arg Ala Arg Ala Ser Ala Ala Leu His Asn Ile Ile 380 385 390	1206
CAC TCA CAG CCT GAT GAC AAG AGA GGC AGG CGT GAA ATC CGA GTC CTT His Ser Gln Pro Asp Asp Lys Arg Gly Arg Arg Glu Ile Arg Val Leu 395 400 405	1254
CAT CTT TTG GAA CAG ATA CGC GCT TAC TGT GAA ACC TGT TGG GAG TGG His Leu Leu Glu Gln Ile Arg Ala Tyr Cys Glu Thr Cys Trp Glu Trp 410 415 420	1302
CAG GAA GCT CAT GAA CCA GGC ATG GAC CAG GAC AAA AAT CCA ATG CCA Gln Glu Ala His Glu Pro Gly Met Asp Gln Asp Lys Asn Pro Met Pro 425 430 435	1350

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GCT Ala 440	CCT Pro	GTT Val	GAA Glu	CAT His	CAG Gln 445	ATC Ile	TGT Cys	CCT Pro	GCT Ala	GTG Val 450	TGT Cys	GTT Val	CTA Leu	ATG Met	AAA Lys 455	1398
CTT Leu	TCA Ser	TTT Phe	GAT Asp 460	GAA Glu 460	GAG Glu	CAT His	AGA Arg	CAT His	GCA Ala 465	ATG Met	AAT Asn	GAA Glu	CTA Leu	GGG Gly 470	GGA Gly	1446
CTA Leu	CAG Gln	GCC Ala	ATT Ile 475	GCA Ala	GAA Glu	TTA Leu	TTG Leu	CAA Gln 480	GTG Val	GAC Asp	TGT Cys	GAA Glu	ATG Met 485	TAT Tyr	GGG Gly	1494
CTT Leu	ACT Thr	AAT Asn 490	GAC Asp	CAC His	TAC Tyr	AGT Ser	ATT Ile 495	ACA Thr	CTA Leu	AGA Arg	CGA Arg	TAT Tyr 500	GCT Ala	GGA Gly	ATG Met	1542
GCT Ala 505	TTG Leu	ACA Thr	AAC Asn	TTG Leu	ACT Thr	TTT Phe 510	GGA Gly	GAT Asp	GTA Val	GCC Ala	AAC Asn 515	AAG Lys	GCT Ala	ACG Thr	CTA Leu	1590
TGC Cys 520	TCT Ser	ATG Met	AAA Lys	GGC Gly	TGC Cys 525	ATG Met	AGA Arg	GCA Ala	CTT Leu	GTG Val 530	GCC Ala	CAA Gln	CTA Leu	AAA Lys	TCT Ser 535	1638
GAA Glu	AGT Ser	GAA Glu	GAC Asp 540	TTA Leu	CAG Gln	CAG Gln	GTT Val	ATT Ile	GCA Ala 545	AGT Ser	GTT Val	TTG Leu	AGG Arg	AAT Asn 550	TTG Leu	1686
TCT Ser	TGG Trp	CGA Arg	GCA Ala 555	GAT Asp	GTA Val	AAT Asn	AGT Ser	AAA Lys 560	AAG Lys	ACG Thr	TTG Leu	CGA Arg	GAA Glu 565	GTT Val	GGA Gly	1734
AGT Ser	GTG Val	AAA Lys 570	GCA Ala	TTG Leu	ATG Met	GAA Glu	TGT Cys 575	GCT Ala	TTA Leu	GAA Glu	GTT Val	AAA Lys 580	AAG Lys	GAA Glu	TCA Ser	1782
ACC Thr	CTC Leu	AAA Lys 585	AGC Ser	GTA Val	TTG Leu	AGT Ser 590	GCC Ala	TTA Leu	TGG Trp	AAT Asn 595	TTG Leu	TCA Ser	GCA Ala	CAT His	TGC Cys	1830
ACT Thr 600	GAG Glu	AAT Asn	AAA Lys	GCT Ala	GAT Asp 605	ATA Ile	TGT Cys	GCT Ala	GTA Val	GAT Asp 610	GGT Gly	GCA Ala	CTT Leu	GCA Ala	TTT Phe 615	1878
TTG Leu	GTT Val	GGC Gly	ACT Thr 620	CTT Leu	ACT Thr	TAC Tyr	CGG Arg	AGC Ser	CAG Gln 625	ACA Thr	AAC Asn	ACT Thr	TTA Leu	GCC Ala 630	ATT Ile	1926
ATT Ile	GAA Glu	AGT Ser	GGA Gly 635	GGT Gly	GGG Gly	ATA Ile	TTA Leu	CGG Arg 640	AAT Asn	GTG Val	TCC Ser	AGC Ser	TTG Leu 645	ATA Ile	GCT Ala	1974
ACA Thr	AAT Asn	GAG Glu	GAC Asp 650	CAC His	AGG Arg	CAA Gln	ATC Ile 655	CTA Leu	AGA Arg	GAG Glu	AAC Asn	AAC Asn 660	TGT Cys	CTA Leu	CAA Gln	2022
ACT Thr	TTA Leu	TTA Leu	CAA Gln	CAC His	TTA Leu	AAA Lys 670	TCT Ser	CAT His	AGT Ser	TTG Leu	ACA Thr 675	ATA Ile	GTC Val	AGT Ser	AAT Asn	2070

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GCA	TGT	GGA	ACT	TTG	TGG	AAT	CTC	TCA	GCA	AGA	AAT	CCT	AAA	GAC	CAG	2118
Ala	Cys	Gly	Thr	Leu	Trp	Asn	Leu	Ser	Ala	Arg	Asn	Pro	Lys	Asp	Gln	
680					685					690					695	
GAA	GCA	TTA	TGG	GAC	ATG	GGG	GCA	GTT	AGC	ATG	CTC	AAG	AAC	CTC	ATT	2166
Glu	Ala	Leu	Trp	Asp	Met	Gly	Ala	Val	Ser	Met	Leu	Lys	Asn	Leu	Ile	
				700					705					710		
CAT	TCA	AAG	CAC	AAA	ATG	ATT	GCT	ATG	GGA	AGT	GCT	GCA	GCT	TTA	AGG	2214
His	Ser	Lys	His	Lys	Met	Ile	Ala	Met	Gly	Ser	Ala	Ala	Ala	Leu	Arg	
			715					720					725			
AAT	CTC	ATG	GCA	AAT	AGG	CCT	GCG	AAG	TAC	AAG	GAT	GCC	AAT	ATT	ATG	2262
Asn	Leu	Met	Ala	Asn	Arg	Pro	Ala	Lys	Tyr	Lys	Asp	Ala	Asn	Ile	Met	
		730					735					740				
TCT	CCT	GGC	TCA	AGC	TTG	CCA	TCT	CTT	CAT	GTT	AGG	AAA	CAA	AAA	GCC	2310
Ser	Pro	Gly	Ser	Ser	Leu	Pro	Ser	Leu	His	Val	Arg	Lys	Gln	Lys	Ala	
	745					750					755					
CTA	GAA	GCA	GAA	TTA	GAT	GCT	CAG	CAC	TTA	TCA	GAA	ACT	TTT	GAC	AAT	2358
Leu	Glu	Ala	Glu	Leu	Asp	Ala	Gln	His	Leu	Ser	Glu	Thr	Phe	Asp	Asn	
760					765					770					775	
ATA	GAC	AAT	TTA	AGT	CCC	AAG	GCA	TCT	CAT	CGT	AGT	AAG	CAG	AGA	CAC	2406
Ile	Asp	Asn	Leu	Ser	Pro	Lys	Ala	Ser	His	Arg	Ser	Lys	Gln	Arg	His	
				780					785					790		
AAG	CAA	AGT	CTC	TAT	GGT	GAT	TAT	GTT	TTT	GAC	ACC	AAT	CGA	CAT	GAT	2454
Lys	Gln	Ser	Leu	Tyr	Gly	Asp	Tyr	Val	Phe	Asp	Thr	Asn	Arg	His	Asp	
			795					800					805			
GAT	AAT	AGG	TCA	GAC	AAT	TTT	AAT	ACT	GGC	AAC	ATG	ACT	GTC	CTT	TCA	2502
Asp	Asn	Arg	Ser	Asp	Asn	Phe	Asn	Thr	Gly	Asn	Met	Thr	Val	Leu	Ser	
		810					815					820				
CCA	TAT	TTG	AAT	ACT	ACA	GTG	TTA	CCC	AGC	TCC	TCT	TCA	TCA	AGA	GGA	2550
Pro	Tyr	Leu	Asn	Thr	Thr	Val	Leu	Pro	Ser	Ser	Ser	Ser	Ser	Arg	Gly	
	825					830					835					
AGC	TTA	GAT	AGT	TCT	CGT	TCT	GAA	AAA	GAT	AGA	AGT	TTG	GAG	AGA	GAA	2598
Ser	Leu	Asp	Ser	Ser	Arg	Ser	Glu	Lys	Asp	Arg	Ser	Leu	Glu	Arg	Glu	
840					845					850					855	
CGC	GGA	ATT	GGT	CTA	GGC	AAC	TAC	CAT	CCA	GCA	ACA	GAA	AAT	CCA	GGA	2646
Arg	Gly	Ile	Gly	Leu	Gly	Asn	Tyr	His	Pro	Ala	Thr	Glu	Asn	Pro	Gly	
			860						865					870		
ACT	TCT	TCA	AAG	CGA	GGT	TTG	CAG	ATC	TCC	ACC	ACT	GCA	GCC	CAG	ATT	2694
Thr	Ser	Ser	Lys	Arg	Gly	Leu	Gln	Ile	Ser	Thr	Thr	Ala	Ala	Gln	Ile	
			875					880					885			
GCC	AAA	GTC	ATG	GAA	GAA	GTG	TCA	GCC	ATT	CAT	ACC	TCT	CAG	GAA	GAC	2742
Ala	Lys	Val	Met	Glu	Glu	Val	Ser	Ala	Ile	His	Thr	Ser	Gln	Glu	Asp	
		890					895					900				
AGA	AGT	TCT	GGG	TCT	ACC	ACT	GAA	TTA	CAT	TGT	GTG	ACA	GAT	GAG	AGA	2790
Arg	Ser	Ser	Gly	Ser	Thr	Thr	Glu	Leu	His	Cys	Val	Thr	Asp	Glu	Arg	
	905					910					915					

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AAT GCA CTT AGA AGA AGC TCT GCT GCC CAT ACA CAT TCA AAC ACT TAC Asn Ala Leu Arg Arg Ser Ser Ala Ala His Thr His Ser Asn Thr Tyr 920 925 930 935	2838
AAT TTC ACT AAG TCG GAA AAT TCA AAT AGG ACA TGT TCT ATG CCT TAT Asn Phe Thr Lys Ser Glu Asn Ser Asn Arg Thr Cys Ser Met Pro Tyr 940 945 950	2886
GCC AAA TTA GAA TAC AAG AGA TCT TCA AAT GAT AGT TTA AAT AGT GTC Ala Lys Leu Glu Tyr Lys Arg Ser Ser Asn Asp Ser Leu Asn Ser Val 955 960 965	2934
AGT AGT AAT GAT GGT TAT GGT AAA AGA GGT CAA ATG AAA CCC TCG ATT Ser Ser Asn Asp Gly Tyr Gly Lys Arg Gly Gln Met Lys Pro Ser Ile 970 975 980	2982
GAA TCC TAT TCT GAA GAT GAT GAA AGT AAG TTT TGC AGT TAT GGT CAA Glu Ser Tyr Ser Glu Asp Asp Glu Ser Lys Phe Cys Ser Tyr Gly Gln 985 990 995	3030
TAC CCA GCC GAC CTA GCC CAT AAA ATA CAT AGT GCA AAT CAT ATC GAT Tyr Pro Ala Asp Leu Ala His Lys Ile His Ser Ala Asn His Met Asp 1000 1005 1010 1015	3078
GAT AAT GAT GGA GAA CTA GAT ACA CCA ATA AAT TAT AGT CTT AAA TAT Asp Asn Asp Gly Glu Leu Asp Thr Pro Ile Asn Tyr Ser Leu Lys Tyr 1020 1025 1030	3126
TCA GAT GAG CAG TTG AAC TCT GGA AGG CAA AGT CCT TCA CAG AAT GAA Ser Asp Glu Gln Leu Asn Ser Gly Arg Gln Ser Pro Ser Gln Asn Glu 1035 1040 1045	3174
AGA TGG GCA AGA CCC AAA CAC ATA ATA GAA GAT GAA ATA AAA CAA AGT Arg Trp Ala Arg Pro Lys His Ile Ile Glu Asp Glu Ile Lys Gln Ser 1050 1055 1060	3222
GAG CAA AGA CAA TCA AGG AAT CAA AGT ACA ACT TAT CCT GTT TAT ACT Glu Gln Arg Gln Ser Arg Asn Gln Ser Thr Thr Tyr Pro Val Tyr Thr 1065 1070 1075	3270
GAG AGC ACT GAT GAT AAA CAC CTC AAG TTC CAA CCA CAT TTT GGA CAG Glu Ser Thr Asp Asp Lys His Leu Lys Phe Gln Pro His Phe Gly Gln 1080 1085 1090 1095	3318
CAG GAA TGT GTT TCT CCA TAC AGG TCA CGG GGA GCC AAT GGT TCA GAA Gln Glu Cys Val Ser Pro Tyr Arg Ser Arg Gly Ala Asn Gly Ser Glu 1100 1105 1110	3366
ACA AAT CGA GTG GGT TCT AAT CAT GGA ATT AAT CAA AAT GTA AGC CAG Thr Asn Arg Val Gly Ser Asn His Gly Ile Asn Gln Asn Val Ser Gln 1115 1120 1125	3414
TCT TTG TGT CAA GAA GAT GAC TAT GAA GAT GAT AAG CCT ACC AAT TAT Ser Leu Cys Gln Glu Asp Asp Tyr Glu Asp Asp Lys Pro Thr Asn Tyr 1130 1135 1140	3462
AGT GAA CGT TAC TCT GAA GAA GAA CAG CAT GAA GAA GAA GAG AGA CCA Ser Glu Arg Tyr Ser Glu Glu Glu Gln His Glu Glu Glu Glu Arg Pro 1145 1150 1155	3510

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ACA AAT TAT AGC ATA AAA TAT AAT GAA GAG AAA CCT CAT GTG GAT CAG Thr Asn Tyr Ser Ile Lys Tyr Asn Glu Glu Lys Arg His Val Asp Gln 1160 1165 1170 1175	3558
CCT ATT GAT TAT AGT TTA AAA TAT GCC ACA GAT ATT CCT TCA TCA CAG Pro Ile Asp Tyr Ser Leu Lys Tyr Ala Thr Asp Ile Pro Ser Ser Gln 1180 1185 1190	3606
AAA CAG TCA TTT TCA TTC TCA AAG AGT TCA TCT GGA CAA AGC AGT AAA Lys Gln Ser Phe Ser Phe Ser Lys Ser Ser Ser Gly Gln Ser Ser Lys 1195 1200 1205	3654
ACC GAA CAT ATG TCT TCA AGC AGT GAG AAT ACG TCC ACA CCT TCA TCT Thr Glu His Met Ser Ser Ser Ser Glu Asn Thr Ser Thr Pro Ser Ser 1210 1215 1220	3702
AAT GCC AAG AGG CAG AAT CAG CTC CAT CCA AGT TCT GCA CAG AGT AGA Asn Ala Lys Arg Gln Asn Gln Leu His Pro Ser Ser Ala Gln Ser Arg 1225 1230 1235	3750
AGT GGT CAG CCT CAA AAG GCT GCC ACT TGC AAA GTT TCT TCT ATT AAC Ser Gly Gln Pro Gln Lys Ala Ala Thr Cys Lys Val Ser Ser Ile Asn 1240 1245 1250 1255	3798
CAA GAA ACA ATA CAG ACT TAT TGT GTA GAA GAT ACT CCA ATA TGT TTT Gln Glu Thr Ile Gln Thr Tyr Cys Val Glu Asp Thr Pro Ile Cys Phe 1260 1265 1270	3846
TCA AGA TGT AGT TCA TTA TCA TCT TTG TCA TCA GCT GAA GAT GAA ATA Ser Arg Cys Ser Ser Leu Ser Ser Leu Ser Ser Ala Glu Asp Glu Ile 1275 1280 1285	3894
GGA TGT AAT CAG ACG ACA CAG GAA GCA GAT TCT GCT AAT ACC CTG CAA Gly Cys Asn Gln Thr Thr Gln Glu Ala Asp Ser Ala Asn Thr Leu Gln 1290 1295 1300	3942
ATA GCA GAA ATA AAA GGA AAG ATT GGA ACT AGG TCA GCT GAA GAT CCT Ile Ala Glu Ile Lys Gly Lys Ile Gly Thr Arg Ser Ala Glu Asp Pro 1305 1310 1315	3990
GTG AGC GAA GTT CCA GCA GTG TCA CAG CAC CCT AGA ACC AAA TCC AGC Val Ser Glu Val Pro Ala Val Ser Gln His Pro Arg Thr Lys Ser Ser 1320 1325 1330 1335	4038
AGA CTG CAG GGT TCT AGT TTA TCT TCA GAA TCA GCC ACG CAC AAA GCT Arg Leu Gln Gly Ser Ser Leu Ser Ser Glu Ser Ala Arg His Lys Ala 1340 1345 1350	4086
GTT GAA TTT CCT TCA GGA GCG AAA TCT CCC TCC AAA AGT GGT GCT CAG Val Glu Phe Pro Ser Gly Ala Lys Ser Pro Ser Lys Ser Gly Ala Gln 1355 1360 1365	4134
ACA CCC AAA AGT CCA CCT GAA CAC TAT GTT CAG GAG ACC CCA CTC ATG Thr Pro Lys Ser Pro Pro Glu His Tyr Val Gln Glu Thr Pro Leu Met 1370 1375 1380	4182
TTT AGC AGA TGT ACT TCT GTC AGT TCA CTT GAT AGT TTT GAG AGT CGT Phe Ser Arg Cys Thr Ser Val Ser Ser Leu Asp Ser Phe Glu Ser Arg 1385 1390 1395	4230



TCG ATT GCC AGC TCC GTT CAG AGT GAA CCA TGC AGT GGA ATG GTA AGT Ser Ile Ala Ser Ser Val Gln Ser Glu Pro Cys Ser Gly Met Val Ser 1400 1405 1410 1415	4278
GGC ATT ATA AGC CCC AGT GAT CTT CCA GAT AGC CCT GGA CAA ACC ATG Gly Ile Ile Ser Pro Ser Asp Leu Pro Asp Ser Pro Gly Gln Thr Met 1420 1425 1430	4326
CCA CCA AGC AGA AGT AAA ACA CCT CCA CCA CCT CCT CAA ACA GCT CAA Pro Pro Ser Arg Ser Lys Thr Pro Pro Pro Pro Pro Gln Thr Ala Gln 1435 1440 1445	4374
ACC AAG CGA GAA GTA CCT AAA AAT AAA GCA CCT ACT GCT GAA AAG AGA Thr Lys Arg Glu Val Pro Lys Asn Lys Ala Pro Thr Ala Glu Lys Arg 1450 1455 1460	4422
GAG AGT GGA CCT AAG CAA GCT GCA GTA AAT GCT GCA GTT CAG AGG GTC Glu Ser Gly Pro Lys Gln Ala Ala Val Asn Ala Ala Val Gln Arg Val 1465 1470 1475	4470
CAG GTT CTT CCA GAT GCT GAT ACT TTA TTA CAT TTT GCC ACA GAA AGT Gln Val Leu Pro Asp Ala Asp Thr Leu Leu His Phe Ala Thr Glu Ser 1480 1485 1490 1495	4518
ACT CCA GAT GGA TTT TCT TGT TCA TCC AGC CTG AGT GCT CTG AGC CTC Thr Pro Asp Gly Phe Ser Cys Ser Ser Ser Leu Ser Ala Leu Ser Leu 1500 1505 1510	4566
GAT GAG CCA TTT ATA CAG AAA GAT GTG GAA TTA AGA ATA ATG CCT CCA Asp Glu Pro Phe Ile Gln Lys Asp Val Glu Leu Arg Ile Met Pro Pro 1515 1520 1525	4614
GTT CAG GAA AAT GAC AAT GGG AAT GAA ACA GAA TCA GAG CAG CCT AAA Val Gln Glu Asn Asp Asn Gly Asn Glu Thr Glu Ser Glu Gln Pro Lys 1530 1535 1540	4662
GAA TCA AAT GAA AAC CAA GAG AAA GAG GCA GAA AAA ACT ATT GAT TCT Glu Ser Asn Glu Asn Gln Glu Lys Glu Ala Glu Lys Thr Ile Asp Ser 1545 1550 1555	4710
GAA AAG GAC CTA TTA GAT GAT TCA GAT GAT GAT GAT ATT GAA ATA CTA Glu Lys Asp Leu Leu Asp Asp Ser Asp Asp Asp Asp Ile Glu Ile Leu 1560 1565 1570 1575	4758
GAA GAA TGT ATT ATT TCT GCC ATG CCA ACA AAG TCA TCA CGT AAA GGC Glu Glu Cys Ile Ile Ser Ala Met Pro Thr Lys Ser Ser Arg Lys Gly 1580 1585 1590	4806
AAA AAG CCA GCC CAG ACT GCT TCA AAA TTA CCT CCA CCT GTG GCA AGG Lys Lys Pro Ala Gln Thr Ala Ser Lys Leu Pro Pro Pro Val Ala Arg 1595 1600 1605	4854
AAA CCA AGT CAG CTG CCT GTG TAC AAA CTT CTA CCA TCA CAA AAC AGG Lys Pro Ser Gln Leu Pro Val Tyr Lys Leu Leu Pro Ser Gln Asn Arg 1610 1615 1620	4902
TTG CAA CCC CAA AAG CAT GTT AGT TTT ACA CCG GGG GAT GAT ATG CCA Leu Gln Pro Gln Lys His Val Ser Phe Thr Pro Gly Asp Asp Met Pro 1625 1630 1635	4950

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CGG GTG TAT TGT GTT GAA GGG ACA CCT ATA AAC TTT TCC ACA GCT ACA Arg Val Tyr Cys Val Glu Gly Thr Pro Ile Asn Phe Ser Thr Ala Thr 1640 1645 1650 1655	4998
TCT CTA AGT GAT CTA ACA ATC GAA TCC CCT CCA AAT GAG TTA GCT GCT Ser Leu Ser Asp Leu Thr Ile Glu Ser Pro Pro Asn Glu Leu Ala Ala 1660 1665 1670	5046
GGA GAA GGA GTT AGA GGA GGA GCA CAG TCA GGT GAA TTT GAA AAA CGA Gly Glu Gly Val Arg Gly Gly Ala Gln Ser Gly Glu Phe Glu Lys Arg 1675 1680 1685	5094
GAT ACC ATT CCT ACA GAA GGC AGA AGT ACA GAT GAG GCT CAA GGA GGA Asp Thr Ile Pro Thr Glu Gly Arg Ser Thr Asp Glu Ala Gln Gly Gly 1690 1695 1700	5142
AAA ACC TCA TCT GTA ACC ATA CCT GAA TTG GAT GAC AAT AAA GCA GAG Lys Thr Ser Ser Val Thr Ile Pro Glu Leu Asp Asn Lys Ala Glu 1705 1710 1715	5190
GAA GGT GAT ATT CTT GCA GAA TGC ATT AAT TCT GCT ATG CCC AAA GGG Glu Gly Asp Ile Leu Ala Glu Cys Ile Asn Ser Ala Met Pro Lys Gly 1720 1725 1730 1735	5238
AAA AGT CAC AAG CCT TTC CGT GTG AAA AAG ATA ATG GAC CAG GTC CAG Lys Ser His Lys Pro Phe Arg Val Lys Lys Ile Met Asp Gln Val Gln 1740 1745 1750	5286
CAA GCA TCT GCG TCG TCT TCT GCA CCC AAC AAA AAT CAG TTA GAT GGT Gln Ala Ser Ala Ser Ser Ser Ala Pro Asn Lys Asn Gln Leu Asp Gly 1755 1760 1765	5334
AAG AAA AAG AAA CCA ACT TCA CCA GTA AAA CCT ATA CCA CAA AAT ACT Lys Lys Lys Lys Pro Thr Ser Pro Val Lys Pro Ile Pro Gln Asn Thr 1770 1775 1780	5382
GAA TAT AGG ACA CGT GTA AGA AAA AAT GCA GAC TCA AAA AAT AAT TTA Glu Tyr Arg Thr Arg Val Arg Lys Asn Ala Asp Ser Lys Asn Asn Leu 1785 1790 1795	5430
AAT GCT GAG AGA GTT TTC TCA GAC AAC AAA GAT TCA AAG AAA CAG AAT Asn Ala Glu Arg Val Phe Ser Asp Asn Lys Asp Ser Lys Lys Gln Asn 1800 1805 1810 1815	5478
TTG AAA AAT AAT TCC AAG GAC TTC AAT GAT AAG CTC CCA AAT AAT GAA Leu Lys Asn Asn Ser Lys Asp Phe Asn Asp Lys Leu Pro Asn Asn Glu 1820 1825 1830	5526
GAT AGA GTC AGA GGA AGT TTT GCT TTT GAT TCA CCT CAT CAT TAC ACG Asp Arg Val Arg Gly Ser Phe Ala Phe Asp Ser Pro His His Tyr Thr 1835 1840 1845	5574
CCT ATT GAA GGA ACT CCT TAC TGT TTT TCA CGA AAT GAT TCT TTG AGT Pro Ile Glu Gly Thr Pro Tyr Cys Phe Ser Arg Asn Asp Ser Leu Ser 1850 1855 1860	5622
TCT CTA GAT TTT GAT GAT GAT GAT GTT GAC CTT TCC AGG GAA AAG GCT Ser Leu Asp Phe Asp Asp Asp Asp Val Asp Leu Ser Arg Glu Lys Ala 1865 1870 1875	5670

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GAA TTA AGA AAG GCA AAA GAA AAT AAG GAA TCA GAG GCT AAA GTT ACC Glu Leu Arg Lys Ala Lys Glu Asn Lys Glu Ser Glu Ala Lys Val Thr 1880 1885 1890 1895	5718
AGC CAC ACA GAA CTA ACC TCC AAC CAA CAA TCA GCT AAT AAG ACA CAA Ser His Thr Glu Leu Thr Ser Asn Gln Gln Ser Ala Asn Lys Thr Gln 1900 1905 1910	5766
GCT ATT GCA AAG CAG CCA ATA AAT CGA GGT CAG CCT AAA CCC ATA CTT Ala Ile Ala Lys Gln Pro Ile Asn Arg Gly Gln Pro Lys Pro Ile Leu 1915 1920 1925	5814
CAG AAA CAA TCC ACT TTT CCC CAG TCA TCC AAA GAC ATA CCA GAC AGA Gln Lys Gln Ser Thr Phe Pro Gln Ser Ser Lys Asp Ile Pro Asp Arg 1930 1935 1940	5862
GGG GCA GCA ACT GAT GAA AAG TTA CAG AAT TTT GCT ATT GAA AAT ACT Gly Ala Ala Thr Asp Glu Lys Leu Gln Asn Phe Ala Ile Glu Asn Thr 1945 1950 1955	5910
CCA GTT TGC TTT TCT CAT AAT TCC TCT CTG AGT TCT CTC AGT GAC ATT Pro Val Cys Phe Ser His Asn Ser Ser Leu Ser Ser Leu Ser Asp Ile 1960 1965 1970 1975	5958
GAC CAA GAA AAC AAC AAT AAA GAA AAT GAA CCT ATC AAA GAG ACT GAG Asp Gln Glu Asn Asn Asn Lys Glu Asn Glu Pro Ile Lys Glu Thr Glu 1980 1985 1990	6006
CCC CCT GAC TCA CAG GGA GAA CCA AGT AAA CCT CAA GCA TCA GGC TAT Pro Pro Asp Ser Gln Gly Glu Pro Ser Lys Pro Gln Ala Ser Gly Tyr 1995 2000 2005	6054
GCT CCT AAA TCA TTT CAT GTT GAA GAT ACC CCA GTT TGT TTC TCA AGA Ala Pro Lys Ser Phe His Val Glu Asp Thr Pro Val Cys Phe Ser Arg 2010 2015 2020	6102
AAC AGT TCT CTC AGT TCT CTT AGT ATT GAC TCT GAA GAT GAC CTG TTG Asn Ser Ser Leu Ser Ser Leu Ser Ile Asp Ser Glu Asp Asp Leu Leu 2025 2030 2035	6150
CAG GAA TGT ATA AGC TCC GCA ATG CCA AAA AAG AAA AAG CCT TCA AGA Gln Glu Cys Ile Ser Ser Ala Met Pro Lys Lys Lys Lys Pro Ser Arg 2040 2045 2050 2055	6198
CTC AAG GGT GAT AAT GAA AAA CAT AGT CCC AGA AAT ATG GGT GGC ATA Leu Lys Gly Asp Asn Glu Lys His Ser Pro Arg Asn Met Gly Gly Ile 2060 2065 2070	6246
TTA GGT GAA GAT CTG ACA CTT GAT TTG AAA GAT ATA CAG AGA CCA GAT Leu Gly Glu Asp Leu Thr Leu Asp Leu Lys Asp Ile Gln Arg Pro Asp 2075 2080 2085	6294
TCA GAA CAT GGT CTA TCC CCT GAT TCA GAA AAT TTT GAT TGG AAA GCT Ser Glu His Gly Leu Ser Pro Asp Ser Glu Asn Phe Asp Trp Lys Ala 2090 2095 2100	6342
ATT CAG GAA GGT GCA AAT TCC ATA GTA AGT AGT TTA CAT CAA GCT GCT Ile Gln Glu Gly Ala Asn Ser Ile Val Ser Ser Leu His Gln Ala Ala 2105 2110 2115	6390

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GCT GCT GCA TGT TTA TCT AGA CAA GCT TCG TCT GAT TCA GAT TCC ATC Ala Ala Ala Cys Leu Ser Arg Gln Ala Ser Ser Asp Ser Asp Ser Ile 2120 2125 2130 2135	6438
CTT TCC CTG AAA TCA GGA ATC TCT CTG GGA TCA CCA TTT CAT CTT ACA Leu Ser Leu Lys Ser Gly Ile Ser Leu Gly Ser Pro Phe His Leu Thr 2140 2145 2150	6486
CCT GAT CAA GAA GAA AAA CCC TTT ACA AGT AAT AAA GGC CCA CGA ATT Pro Asp Gln Glu Glu Lys Pro Phe Thr Ser Asn Lys Gly Pro Arg Ile 2155 2160 2165	6534
CTA AAA CCA GGG GAG AAA AGT ACA TTG GAA ACT AAA AAG ATA GAA TCT Leu Lys Pro Gly Glu Lys Ser Thr Leu Glu Thr Lys Lys Ile Glu Ser 2170 2175 2180	6582
GAA AGT AAA GGA ATC AAA GGA GGA AAA AAA GTT TAT AAA AGT TTG ATT Glu Ser Lys Gly Ile Lys Gly Gly Lys Lys Val Tyr Lys Ser Leu Ile 2185 2190 2195	6630
ACT GGA AAA GTT CGA TCT AAT TCA GAA ATT TCA GGC CAA ATG AAA CAG Thr Gly Lys Val Arg Ser Asn Ser Glu Ile Ser Gly Gln Met Lys Gln 2200 2205 2210 2215	6678
CCC CTT CAA GCA AAC ATG CCT TCA ATC TCT CGA GGC AGG ACA ATG ATT Pro Leu Gln Ala Asn Met Pro Ser Ile Ser Arg Gly Arg Thr Met Ile 2220 2225 2230	6726
CAT ATT CCA GGA GTT CGA AAT AGC TCC TCA AGT ACA AGT CCT GTT TCT His Ile Pro Gly Val Arg Asn Ser Ser Ser Ser Thr Ser Pro Val Ser 2235 2240 2245	6774
AAA AAA GGC CCA CCC CTT AAG ACT CCA GCC TCC AAA AGC CCT AGT GAA Lys Lys Gly Pro Pro Leu Lys Thr Pro Ala Ser Lys Ser Pro Ser Glu 2250 2255 2260	6822
GGT CAA ACA GCC ACC ACT TCT CCT AGA GGA GCC AAG CCA TCT GTG AAA Gly Gln Thr Ala Thr Thr Ser Pro Arg Gly Ala Lys Pro Ser Val Lys 2265 2270 2275	6870
TCA GAA TTA AGC CCT GTT GCC AGG CAG ACA TCC CAA ATA GGT GGG TCA Ser Glu Leu Ser Pro Val Ala Arg Gln Thr Ser Gln Ile Gly Gly Ser 2280 2285 2290 2295	6918
AGT AAA GCA CCT TCT AGA TCA GGA TCT AGA GAT TCG ACC CCT TCA AGA Ser Lys Ala Pro Ser Arg Ser Gly Ser Arg Asp Ser Thr Pro Ser Arg 2300 2305 2310	6966
CCT GCC CAG CAA CCA TTA AGT AGA CCT ATA CAG TCT CCT GGC CGA AAC Pro Ala Gln Gln Pro Leu Ser Arg Pro Ile Gln Ser Pro Gly Arg Asn 2315 2320 2325	7014
TCA ATT TCC CCT GGT AGA AAT GGA ATA AGT CCT CCT AAC AAA TTA TCT Ser Ile Ser Pro Gly Arg Asn Gly Ile Ser Pro Pro Asn Lys Leu Ser 2330 2335 2340	7062
CAA CTT CCA AGG ACA TCA TCC CCT AGT ACT GCT TCA ACT AAG TCC TCA Gln Leu Pro Arg Thr Ser Ser Pro Ser Thr Ala Ser Thr Lys Ser Ser 2345 2350 2355	7110

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GGT TCT GGA AAA ATG TCA TAT ACA TCT CCA GGT AGA CAG ATG AGC CAA Gly Ser Gly Lys Met Ser Tyr Thr Ser Pro Gly Arg Gln Met Ser Gln 2360 2365 2370 2375	7158
CAG AAC CTT ACC AAA CAA ACA GGT TTA TCC AAG AAT GCC AGT AGT ATT Gln Asn Leu Thr Lys Gln Thr Gly Leu Ser Lys Asn Ala Ser Ser Ile 2380 2385 2390	7206
CCA AGA AGT GAG TCT GCC TCC AAA GGA CTA AAT CAG ATG AAT AAT GGT Pro Arg Ser Glu Ser Ala Ser Lys Gly Leu Asn Gln Met Asn Asn Gly 2395 2400 2405	7254
AAT GGA GCC AAT AAA AAG GTA GAA CTT TCT AGA ATG TCT TCA ACT AAA Asn Gly Ala Asn Lys Lys Val Glu Leu Ser Arg Met Ser Ser Thr Lys 2410 2415 2420	7302
TCA AGT GGA AGT GAA TCT GAT AGA TCA GAA AGA CCT GTA TTA GTA CGC Ser Ser Gly Ser Glu Ser Asp Arg Ser Glu Arg Pro Val Leu Val Arg 2425 2430 2435	7350
CAG TCA ACT TTC ATC AAA GAA GCT CCA AGC CCA ACC TTA AGA AGA AAA Gln Ser Thr Phe Ile Lys Glu Ala Pro Ser Pro Thr Leu Arg Arg Lys 2440 2445 2450 2455	7398
TTG GAG GAA TCT GCT TCA TTT GAA TCT CTT TCT CCA TCA TCT AGA CCA Leu Glu Glu Ser Ala Ser Phe Glu Ser Leu Ser Pro Ser Ser Arg Pro 2460 2465 2470	7446
GCT TCT CCC ACT AGG TCC CAG GCA CAA ACT CCA GTT TTA AGT CCT TCC Ala Ser Pro Thr Arg Ser Gln Ala Gln Thr Pro Val Leu Ser Pro Ser 2475 2480 2485	7494
CTT CCT GAT ATG TCT CTA TCC ACA CAT TCG TCT GTT CAG GCT GGT GGA Leu Pro Asp Met Ser Leu Ser Thr His Ser Ser Val Gln Ala Gly Gly 2490 2495 2500	7542
TGG CGA AAA CTC CCA CCT AAT CTC AGT CCC ACT ATA GAG TAT AAT GAT Trp Arg Lys Leu Pro Pro Asn Leu Ser Pro Thr Ile Glu Tyr Asn Asp 2505 2510 2515	7590
GGA AGA CCA GCA AAG CGC CAT GAT ATT GCA CGG TCT CAT TCT GAA AGT Gly Arg Pro Ala Lys Arg His Asp Ile Ala Arg Ser His Ser Glu Ser 2520 2525 2530 2535	7638
CCT TCT AGA CTT CCA ATC AAT AGG TCA GGA ACC TGG AAA CGT GAG CAC Pro Ser Arg Leu Pro Ile Asn Arg Ser Gly Thr Trp Lys Arg Glu His 2540 2545 2550	7686
AGC AAA CAT TCA TCA TCC CTT CCT CGA GTA AGC ACT TGG AGA AGA ACT Ser Lys His Ser Ser Ser Leu Pro Arg Val Ser Thr Trp Arg Arg Thr 2555 2560 2565	7734
GGA AGT TCA TCT TCA ATT CTT TCT GCT TCA TCA GAA TCC AGT GAA AAA Gly Ser Ser Ser Ser Ile Leu Ser Ala Ser Ser Glu Ser Ser Glu Lys 2570 2575 2580	7782
GCA AAA AGT GAG GAT GAA AAA CAT GTG AAC TCT ATT TCA GGA ACC AAA Ala Lys Ser Glu Asp Glu Lys His Val Asn Ser Ile Ser Gly Thr Lys 2585 2590 2595	7830

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CAA AGT AAA GAA AAC CAA GTA TCC GCA AAA GGA ACA TGG AGA AAA ATA Gln Ser Lys Glu Asn Gln Val Ser Ala Lys Gly Thr Trp Arg Lys Ile 2600 2605 2610 2615	7878
AAA GAA AAT GAA TTT TCT CCC ACA AAT AGT ACT TCT CAG ACC GTT TCC Lys Glu Asn Glu Phe Ser Pro Thr Asn Ser Thr Ser Gln Thr Val Ser 2620 2625 2630	7926
TCA GGT GCT ACA AAT GGT GCT GAA TCA AAG ACT CTA ATT TAT CAA ATG Ser Gly Ala Thr Asn Gly Ala Glu Ser Lys Thr Leu Ile Tyr Gln Met 2635 2640 2645	7974
GCA CCT GCT GTT TCT AAA ACA GAG GAT GTT TGG GTG AGA ATT GAG GAC Ala Pro Ala Val Ser Lys Thr Glu Asp Val Trp Val Arg Ile Glu Asp 2650 2655 2660	8022
TGT CCC ATT AAC AAT CCT AGA TCT GGA AGA TCT CCC ACA GGT AAT ACT Cys Pro Ile Asn Asn Pro Arg Ser Gly Arg Ser Pro Thr Gly Asn Thr 2665 2670 2675	8070
CCC CCG GTG ATT GAC AGT GTT TCA GAA AAG GCA AAT CCA AAC ATT AAA Pro Pro Val Ile Asp Ser Val Ser Glu Lys Ala Asn Pro Asn Ile Lys 2680 2685 2690 2695	8118
GAT TCA AAA GAT AAT CAG GCA AAA CAA AAT GTG GGT AAT GGC AGT GTT Asp Ser Lys Asp Asn Gln Ala Lys Gln Asn Val Gly Asn Gly Ser Val 2700 2705 2710	8166
CCC ATG CGT ACC GTG GGT TTG GAA AAT CGC CTG ACC TCC TTT ATT CAG Pro Met Arg Thr Val Gly Leu Glu Asn Arg Leu Thr Ser Phe Ile Gln 2715 2720 2725	8214
GTG GAT GCC CCT GAC CAA AAA GGA ACT GAG ATA AAA CCA GGA CAA AAT Val Asp Ala Pro Asp Gln Lys Gly Thr Glu Ile Lys Pro Gly Gln Asn 2730 2735 2740	8262
AAT CCT GTC CCT GTA TCA GAG ACT AAT GAA AGT CCT ATA GTG GAA CGT Asn Pro Val Pro Val Ser Glu Thr Asn Glu Ser Pro Ile Val Glu Arg 2745 2750 2755	8310
ACC CCA TTC AGT TCT AGC AGC TCA AGC AAA CAC AGT TCA CCT AGT GGG Thr Pro Phe Ser Ser Ser Ser Ser Lys His Ser Ser Pro Ser Gly 2760 2765 2770 2775	8358
ACT GTT GCT GCC AGA GTG ACT CCT TTT AAT TAC AAC CCA AGC CCT AGG Thr Val Ala Ala Arg Val Thr Pro Phe Asn Tyr Asn Pro Ser Pro Arg 2780 2785 2790	8406
AAA AGC AGC GCA GAT AGC ACT TCA GCT CGG CCA TCT CAG ATC CCA ACT Lys Ser Ser Ala Asp Ser Thr Ser Ala Arg Pro Ser Gln Ile Pro Thr 2795 2800 2805	8454
CCA GTG AAT AAC AAC ACA AAG AAG CGA GAT TCC AAA ACT GAC AGC ACA Pro Val Asn Asn Asn Thr Lys Lys Arg Asp Ser Lys Thr Asp Ser Thr 2810 2815 2820	8502
GAA TCC AGT GGA ACC CAA AGT CCT AAG CGC CAT TCT GGG TCT TAC CTT Glu Ser Ser Gly Thr Gln Ser Pro Lys Arg His Ser Gly Ser Tyr Leu 2825 2830 2835	8550

GTG ACA TCT GTT TAAAAGAGAG GAAGAATGAA ACTAAGAAAA TTCTATGTTA 8602  
 Val Thr Ser Val  
 2840

ATTACAACCTG CTATATAGAC ATTTTGTTTC AAATGAAACT TTAAAAGACT GAAAAATTTT 8662

GTAATAGGT TTGATTCTTG TTAGAGGGTT TTTGTTCTGG AAGCCATATT TGATAGTATA 8722

CTTTGTCTTC ACTGGTCTTA TTTGGGAGG CACTCTTGAT GGTTAGGAAA AAATAGAAAG 8782

CCAAGTATGT TTGTACAGTA TGTTTTACAT GTATTTAAAG TAGCATCCCA TCCCAACTTC 8842

CTTAATTATT GCTTGTCTAA AATAATGAAC ACTACAGATA GGAAATATGA TATATTGCTG 8902

TTATCAATCA TTTCTAGATT ATAACTGAC TAACTTACA TCAGGGGAAA ATTGGTATTT 8962

ATGCAAAAAA AAAATGTTTT TGTCTTGTG AGTCCATCTA ACATCATAAT TAATCATGTG 9022

GCTGTGAAAT TCACAGTAAT ATGGTTCCCG ATGAACAAGT TTACCCAGCC TGCTTTGCTT 9082

ACTGCATGAA TGAACTGAT GGTTCATTT CAGAAGTAAT GATTAACACT TATGTGGTCA 9142

CATGATGTGC ATAGAGATAG CTACAGTGTA ATAATTTACA CTATTTTGTG CTCCAAACAA 9202

AACAAAAATC TGTGTAACCTG TAAAACATTG AATGAACTA TTTTACCTGA ACTAGATTTT 9262

ATCTGAAAGT AGGTAGAATT TTTGCTATGC TGTAAATTTGT TGTATATTCT GGTATTTGAG 9322

GTGAGATGGC TGCTCTTTAT TAATGAGACA TGAATTGTGT CTCAACAGAA ACTAAATGAA 9382

CATTTTCAGAA TAAATTATTG CTGTATGTAA ACTGTTACTG AAATTGGTAT TTGTTTGAAG 9442

GGTTTGTTTC ACATTTGTAT TAATTAATTG TTTAAAATGC CTCTTTTAAA AGCTTATATA 9502

AATTTTTTCT TCAGCTTCTA TGCATTAAGA GTAAAATTCC TCTTACTGTA ATAAAAACAT 9562

TGAAGAAGAC TGTGCCACT TAACCATTCC ATGCGTTGGC ACTT 9606

## (2) INFORMATION FOR SEQ ID NO:2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2843 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Ala Ala Ser Tyr Asp Gln Leu Leu Lys Gln Val Glu Ala Leu  
 1 5 10 15

Lys Met Glu Asn Ser Asn Leu Arg Gln Glu Leu Glu Asp Asn Ser Asn  
 20 25 30

His Leu Thr Lys Leu Glu Thr Glu Ala Ser Asn Met Lys Glu Val Leu  
 35 40 45

Lys Gln Leu Gln Gly Ser Ile Glu Asp Glu Ala Met Ala Ser Ser Gly  
 50 55 60

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Gln Ile Asp Leu Leu Glu Arg Leu Lys Glu Leu Asn Leu Asp Ser Ser  
 65 70 75 80  
 Asn Phe Pro Gly Val Lys Leu Arg Ser Lys Met Ser Leu Arg Ser Tyr  
 85 90 95  
 Gly Ser Arg Glu Gly Ser Val Ser Ser Arg Ser Gly Glu Cys Ser Pro  
 100 105 110  
 Val Pro Met Gly Ser Phe Pro Arg Arg Gly Phe Val Asn Gly Ser Arg  
 115 120 125  
 Glu Ser Thr Gly Tyr Leu Glu Leu Glu Lys Glu Arg Ser Leu Leu  
 130 135 140  
 Leu Ala Asp Leu Asp Lys Glu Glu Lys Glu Lys Asp Trp Tyr Tyr Ala  
 145 150 155 160  
 Gln Leu Gln Asn Leu Thr Lys Arg Ile Asp Ser Leu Pro Leu Thr Glu  
 165 170 175  
 Asn Phe Ser Leu Gln Thr Asp Leu Thr Arg Arg Gln Leu Glu Tyr Glu  
 180 185 190  
 Ala Arg Gln Ile Arg Val Ala Met Glu Glu Gln Leu Gly Thr Cys Gln  
 195 200 205  
 Asp Met Glu Lys Arg Ala Gln Arg Arg Ile Ala Arg Ile Gln Gln Ile  
 210 215 220  
 Glu Lys Asp Ile Leu Arg Ile Arg Gln Leu Leu Gln Ser Gln Ala Thr  
 225 230 235 240  
 Glu Ala Glu Arg Ser Ser Gln Asn Lys His Glu Thr Gly Ser His Asp  
 245 250 255  
 Ala Glu Arg Gln Asn Glu Gly Gln Gly Val Gly Glu Ile Asn Met Ala  
 260 265 270  
 Thr Ser Gly Asn Gly Gln Gly Ser Thr Thr Arg Met Asp His Glu Thr  
 275 280 285  
 Ala Ser Val Leu Ser Ser Ser Ser Thr His Ser Ala Pro Arg Arg Leu  
 290 295 300  
 Thr Ser His Leu Gly Thr Lys Val Glu Met Val Tyr Ser Leu Leu Ser  
 305 310 315 320  
 Met Leu Gly Thr His Asp Lys Asp Asp Met Ser Arg Thr Leu Leu Ala  
 325 330 335  
 Met Ser Ser Ser Gln Asp Ser Cys Ile Ser Met Arg Gln Ser Gly Cys  
 340 345 350  
 Leu Pro Leu Leu Ile Gln Leu Leu His Gly Asn Asp Lys Asp Ser Val  
 355 360 365  
 Leu Leu Gly Asn Ser Arg Gly Ser Lys Glu Ala Arg Ala Arg Ala Ser  
 370 375 380  
 Ala Ala Leu His Asn Ile Ile His Ser Gln Pro Asp Asp Lys Arg Gly  
 385 390 395 400

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Arg Arg Glu Ile Arg Val Leu His Leu Leu Glu Gln Ile Arg Ala Tyr  
 405 410 415  
 Cys Glu Thr Cys Trp Glu Trp Gln Glu Ala His Glu Pro Gly Met Asp  
 420 425 430  
 Gln Asp Lys Asn Pro Met Pro Ala Pro Val Glu His Gln Ile Cys Pro  
 435 440 445  
 Ala Val Cys Val Leu Met Lys Leu Ser Phe Asp Glu Glu His Arg His  
 450 455 460  
 Ala Met Asn Glu Leu Gly Gly Leu Gln Ala Ile Ala Glu Leu Leu Gln  
 465 470 475 480  
 Val Asp Cys Glu Met Tyr Gly Leu Thr Asn Asp His Tyr Ser Ile Thr  
 485 490 495  
 Leu Arg Arg Tyr Ala Gly Met Ala Leu Thr Asn Leu Thr Phe Gly Asp  
 500 505 510  
 Val Ala Asn Lys Ala Thr Leu Cys Ser Met Lys Gly Cys Met Arg Ala  
 515 520 525  
 Leu Val Ala Gln Leu Lys Ser Glu Ser Glu Asp Leu Gln Gln Val Ile  
 530 535 540  
 Ala Ser Val Leu Arg Asn Leu Ser Trp Arg Ala Asp Val Asn Ser Lys  
 545 550 555 560  
 Lys Thr Leu Arg Glu Val Gly Ser Val Lys Ala Leu Met Glu Cys Ala  
 565 570 575  
 Leu Glu Val Lys Lys Glu Ser Thr Leu Lys Ser Val Leu Ser Ala Leu  
 580 585 590  
 Trp Asn Leu Ser Ala His Cys Thr Glu Asn Lys Ala Asp Ile Cys Ala  
 595 600 605  
 Val Asp Gly Ala Leu Ala Phe Leu Val Gly Thr Leu Thr Tyr Arg Ser  
 610 615 620  
 Gln Thr Asn Thr Leu Ala Ile Ile Glu Ser Gly Gly Gly Ile Leu Arg  
 625 630 635 640  
 Asn Val Ser Ser Leu Ile Ala Thr Asn Glu Asp His Arg Gln Ile Leu  
 645 650 655  
 Arg Glu Asn Asn Cys Leu Gln Thr Leu Leu Gln His Leu Lys Ser His  
 660 665 670  
 Ser Leu Thr Ile Val Ser Asn Ala Cys Gly Thr Leu Trp Asn Leu Ser  
 675 680 685  
 Ala Arg Asn Pro Lys Asp Gln Glu Ala Leu Trp Asp Met Gly Ala Val  
 690 695 700  
 Ser Met Leu Lys Asn Leu Ile His Ser Lys His Lys Met Ile Ala Met  
 705 710 715 720  
 Gly Ser Ala Ala Ala Leu Arg Asn Leu Met Ala Asn Arg Pro Ala Lys  
 725 730 735

Tyr Lys Asp Ala Asn Ile Met Ser Pro Gly Ser Ser Leu Pro Ser Leu  
 740 745 750  
 His Val Arg Lys Gln Lys Ala Leu Glu Ala Glu Leu Asp Ala Gln His  
 755 760 765  
 Leu Ser Glu Thr Phe Asp Asn Ile Asp Asn Leu Ser Pro Lys Ala Ser  
 770 775 780  
 His Arg Ser Lys Gln Arg His Lys Gln Ser Leu Tyr Gly Asp Tyr Val  
 785 790 795 800  
 Phe Asp Thr Asn Arg His Asp Asp Asn Arg Ser Asp Asn Phe Asn Thr  
 805 810 815  
 Gly Asn Met Thr Val Leu Ser Pro Tyr Leu Asn Thr Thr Val Leu Pro  
 820 825 830  
 Ser Ser Ser Ser Ser Arg Gly Ser Leu Asp Ser Ser Arg Ser Glu Lys  
 835 840 845  
 Asp Arg Ser Leu Glu Arg Glu Arg Gly Ile Gly Leu Gly Asn Tyr His  
 850 855 860  
 Pro Ala Thr Glu Asn Pro Gly Thr Ser Ser Lys Arg Gly Leu Gln Ile  
 865 870 875 880  
 Ser Thr Thr Ala Ala Gln Ile Ala Lys Val Met Glu Glu Val Ser Ala  
 885 890 895  
 Ile His Thr Ser Gln Glu Asp Arg Ser Ser Gly Ser Thr Thr Glu Leu  
 900 905 910  
 His Cys Val Thr Asp Glu Arg Asn Ala Leu Arg Arg Ser Ser Ala Ala  
 915 920 925  
 His Thr His Ser Asn Thr Tyr Asn Phe Thr Lys Ser Glu Asn Ser Asn  
 930 935 940  
 Arg Thr Cys Ser Met Pro Tyr Ala Lys Leu Glu Tyr Lys Arg Ser Ser  
 945 950 955 960  
 Asn Asp Ser Leu Asn Ser Val Ser Ser Asn Asp Gly Tyr Gly Lys Arg  
 965 970 975  
 Gly Gln Met Lys Pro Ser Ile Glu Ser Tyr Ser Glu Asp Asp Glu Ser  
 980 985 990  
 Lys Phe Cys Ser Tyr Gly Gln Tyr Pro Ala Asp Leu Ala His Lys Ile  
 995 1000 1005  
 His Ser Ala Asn His Met Asp Asp Asn Asp Gly Glu Leu Asp Thr Pro  
 1010 1015 1020  
 Ile Asn Tyr Ser Leu Lys Tyr Ser Asp Glu Gln Leu Asn Ser Gly Arg  
 1025 1030 1035 1040  
 Gln Ser Pro Ser Gln Asn Glu Arg Trp Ala Arg Pro Lys His Ile Ile  
 1045 1050 1055  
 Glu Asp Glu Ile Lys Gln Ser Glu Gln Arg Gln Ser Arg Asn Gln Ser  
 1060 1065 1070

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Thr Thr Tyr Pro Val Tyr Thr Glu Ser Thr Asp Asp Lys His Leu Lys  
 1075 1080 1085  
 Phe Gln Pro His Phe Gly Gln Gln Glu Cys Val Ser Pro Tyr Arg Ser  
 1090 1095 1100  
 Arg Gly Ala Asn Gly Ser Glu Thr Asn Arg Val Gly Ser Asn His Gly  
 1105 1110 1115 1120  
 Ile Asn Gln Asn Val Ser Gln Ser Leu Cys Gln Glu Asp Asp Tyr Glu  
 1125 1130 1135  
 Asp Asp Lys Pro Thr Asn Tyr Ser Glu Arg Tyr Ser Glu Glu Glu Gln  
 1140 1145 1150  
 His Glu Glu Glu Glu Arg Pro Thr Asn Tyr Ser Ile Lys Tyr Asn Glu  
 1155 1160 1165  
 Glu Lys Arg His Val Asp Gln Pro Ile Asp Tyr Ser Leu Lys Tyr Ala  
 1170 1175 1180  
 Thr Asp Ile Pro Ser Ser Gln Lys Gln Ser Phe Ser Phe Ser Lys Ser  
 1185 1190 1195 1200  
 Ser Ser Gly Gln Ser Ser Lys Thr Glu His Met Ser Ser Ser Ser Glu  
 1205 1210 1215  
 Asn Thr Ser Thr Pro Ser Ser Asn Ala Lys Arg Gln Asn Gln Leu His  
 1220 1225 1230  
 Pro Ser Ser Ala Gln Ser Arg Ser Gly Gln Pro Gln Lys Ala Ala Thr  
 1235 1240 1245  
 Cys Lys Val Ser Ser Ile Asn Gln Glu Thr Ile Gln Thr Tyr Cys Val  
 1250 1255 1260  
 Glu Asp Thr Pro Ile Cys Phe Ser Arg Cys Ser Ser Leu Ser Ser Leu  
 1265 1270 1275 1280  
 Ser Ser Ala Glu Asp Glu Ile Gly Cys Asn Gln Thr Thr Gln Glu Ala  
 1285 1290 1295  
 Asp Ser Ala Asn Thr Leu Gln Ile Ala Glu Ile Lys Gly Lys Ile Gly  
 1300 1305 1310  
 Thr Arg Ser Ala Glu Asp Pro Val Ser Glu Val Pro Ala Val Ser Gln  
 1315 1320 1325  
 His Pro Arg Thr Lys Ser Ser Arg Leu Gln Gly Ser Ser Leu Ser Ser  
 1330 1335 1340  
 Glu Ser Ala Arg His Lys Ala Val Glu Phe Pro Ser Gly Ala Lys Ser  
 1345 1350 1355 1360  
 Pro Ser Lys Ser Gly Ala Gln Thr Pro Lys Ser Pro Pro Glu His Tyr  
 1365 1370 1375  
 Val Gln Glu Thr Pro Leu Met Phe Ser Arg Cys Thr Ser Val Ser Ser  
 1380 1385 1390  
 Leu Asp Ser Phe Glu Ser Arg Ser Ile Ala Ser Ser Val Gln Ser Glu  
 1395 1400 1405

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Pro Cys Ser Gly Met Val Ser Gly Ile Ile Ser Pro Ser Asp Leu Pro  
 1410 1415 1420  
 Asp Ser Pro Gly Gln Thr Met Pro Pro Ser Arg Ser Lys Thr Pro Pro  
 1425 1430 1435 1440  
 Pro Pro Pro Gln Thr Ala Gln Thr Lys Arg Glu Val Pro Lys Asn Lys  
 1445 1450 1455  
 Ala Pro Thr Ala Glu Lys Arg Glu Ser Gly Pro Lys Gln Ala Ala Val  
 1460 1465 1470  
 Asn Ala Ala Val Gln Arg Val Gln Val Leu Pro Asp Ala Asp Thr Leu  
 1475 1480 1485  
 Leu His Phe Ala Thr Glu Ser Thr Pro Asp Gly Phe Ser Cys Ser Ser  
 1490 1495 1500  
 Ser Leu Ser Ala Leu Ser Leu Asp Glu Pro Phe Ile Gln Lys Asp Val  
 1505 1510 1515 1520  
 Glu Leu Arg Ile Met Pro Pro Val Gln Glu Asn Asp Asn Gly Asn Glu  
 1525 1530 1535  
 Thr Glu Ser Glu Gln Pro Lys Glu Ser Asn Glu Asn Gln Glu Lys Glu  
 1540 1545 1550  
 Ala Glu Lys Thr Ile Asp Ser Glu Lys Asp Leu Leu Asp Asp Ser Asp  
 1555 1560 1565  
 Asp Asp Asp Ile Glu Ile Leu Glu Glu Cys Ile Ile Ser Ala Met Pro  
 1570 1575 1580  
 Thr Lys Ser Ser Arg Lys Gly Lys Lys Pro Ala Gln Thr Ala Ser Lys  
 1585 1590 1595 1600  
 Leu Pro Pro Pro Val Ala Arg Lys Pro Ser Gln Leu Pro Val Tyr Lys  
 1605 1610 1615  
 Leu Leu Pro Ser Gln Asn Arg Leu Gln Pro Gln Lys His Val Ser Phe  
 1620 1625 1630  
 Thr Pro Gly Asp Asp Met Pro Arg Val Tyr Cys Val Glu Gly Thr Pro  
 1635 1640 1645  
 Ile Asn Phe Ser Thr Ala Thr Ser Leu Ser Asp Leu Thr Ile Glu Ser  
 1650 1655 1660  
 Pro Pro Asn Glu Leu Ala Ala Gly Glu Gly Val Arg Gly Gly Ala Gln  
 1665 1670 1675 1680  
 Ser Gly Glu Phe Glu Lys Arg Asp Thr Ile Pro Thr Glu Gly Arg Ser  
 1685 1690 1695  
 Thr Asp Glu Ala Gln Gly Gly Lys Thr Ser Ser Val Thr Ile Pro Glu  
 1700 1705 1710  
 Leu Asp Asp Asn Lys Ala Glu Glu Gly Asp Ile Leu Ala Glu Cys Ile  
 1715 1720 1725  
 Asn Ser Ala Met Pro Lys Gly Lys Ser His Lys Pro Phe Arg Val Lys  
 1730 1735 1740

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Lys Ile Met Asp Gln Val Gln Gln Ala Ser Ala Ser Ser Ser Ala Pro  
 1745 1750 1755 1760  
 Asn Lys Asn Gln Leu Asp Gly Lys Lys Lys Lys Pro Thr Ser Pro Val  
 1765 1770 1775  
 Lys Pro Ile Pro Gln Asn Thr Glu Tyr Arg Thr Arg Val Arg Lys Asn  
 1780 1785 1790  
 Ala Asp Ser Lys Asn Asn Leu Asn Ala Glu Arg Val Phe Ser Asp Asn  
 1795 1800 1805  
 Lys Asp Ser Lys Lys Gln Asn Leu Lys Asn Asn Ser Lys Asp Phe Asn  
 1810 1815 1820  
 Asp Lys Leu Pro Asn Asn Glu Asp Arg Val Arg Gly Ser Phe Ala Phe  
 1825 1830 1835 1840  
 Asp Ser Pro His His Tyr Thr Pro Ile Glu Gly Thr Pro Tyr Cys Phe  
 1845 1850 1855  
 Ser Arg Asn Asp Ser Leu Ser Ser Leu Asp Phe Asp Asp Asp Val  
 1860 1865 1870  
 Asp Leu Ser Arg Glu Lys Ala Glu Leu Arg Lys Ala Lys Glu Asn Lys  
 1875 1880 1885  
 Glu Ser Glu Ala Lys Val Thr Ser His Thr Glu Leu Thr Ser Asn Gln  
 1890 1895 1900  
 Gln Ser Ala Asn Lys Thr Gln Ala Ile Ala Lys Gln Pro Ile Asn Arg  
 1905 1910 1915 1920  
 Gly Gln Pro Lys Pro Ile Leu Gln Lys Gln Ser Thr Phe Pro Gln Ser  
 1925 1930 1935  
 Ser Lys Asp Ile Pro Asp Arg Gly Ala Ala Thr Asp Glu Lys Leu Gln  
 1940 1945 1950  
 Asn Phe Ala Ile Glu Asn Thr Pro Val Cys Phe Ser His Asn Ser Ser  
 1955 1960 1965  
 Leu Ser Ser Leu Ser Asp Ile Asp Gln Glu Asn Asn Asn Lys Glu Asn  
 1970 1975 1980  
 Glu Pro Ile Lys Glu Thr Glu Pro Pro Asp Ser Gln Gly Glu Pro Ser  
 1985 1990 1995 2000  
 Lys Pro Gln Ala Ser Gly Tyr Ala Pro Lys Ser Phe His Val Glu Asp  
 2005 2010 2015  
 Thr Pro Val Cys Phe Ser Arg Asn Ser Ser Leu Ser Ser Leu Ser Ile  
 2020 2025 2030  
 Asp Ser Glu Asp Asp Leu Leu Gln Glu Cys Ile Ser Ser Ala Met Pro  
 2035 2040 2045  
 Lys Lys Lys Lys Pro Ser Arg Leu Lys Gly Asp Asn Glu Lys His Ser  
 2050 2055 2060  
 Pro Arg Asn Met Gly Gly Ile Leu Gly Glu Asp Leu Thr Leu Asp Leu  
 2065 2070 2075 2080

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Lys Asp Ile Gln Arg Pro Asp Ser Glu His Gly Leu Ser Pro Asp Ser  
 2085 2090 2095  
 Glu Asn Phe Asp Trp Lys Ala Ile Gln Glu Gly Ala Asn Ser Ile Val  
 2100 2105 2110  
 Ser Ser Leu His Gln Ala Ala Ala Ala Cys Leu Ser Arg Gln Ala  
 2115 2120 2125  
 Ser Ser Asp Ser Asp Ser Ile Leu Ser Leu Lys Ser Gly Ile Ser Leu  
 2130 2135 2140  
 Gly Ser Pro Phe His Leu Thr Pro Asp Gln Glu Glu Lys Pro Phe Thr  
 2145 2150 2155 2160  
 Ser Asn Lys Gly Pro Arg Ile Leu Lys Pro Gly Glu Lys Ser Thr Leu  
 2165 2170 2175  
 Glu Thr Lys Lys Ile Glu Ser Glu Ser Lys Gly Ile Lys Gly Gly Lys  
 2180 2185 2190  
 Lys Val Tyr Lys Ser Leu Ile Thr Gly Lys Val Arg Ser Asn Ser Glu  
 2195 2200 2205  
 Ile Ser Gly Gln Met Lys Gln Pro Leu Gln Ala Asn Met Pro Ser Ile  
 2210 2215 2220  
 Ser Arg Gly Arg Thr Met Ile His Ile Pro Gly Val Arg Asn Ser Ser  
 2225 2230 2235 2240  
 Ser Ser Thr Ser Pro Val Ser Lys Lys Gly Pro Pro Leu Lys Thr Pro  
 2245 2250 2255  
 Ala Ser Lys Ser Pro Ser Glu Gly Gln Thr Ala Thr Thr Ser Pro Arg  
 2260 2265 2270  
 Gly Ala Lys Pro Ser Val Lys Ser Glu Leu Ser Pro Val Ala Arg Gln  
 2275 2280 2285  
 Thr Ser Gln Ile Gly Gly Ser Ser Lys Ala Pro Ser Arg Ser Gly Ser  
 2290 2295 2300  
 Arg Asp Ser Thr Pro Ser Arg Pro Ala Gln Gln Pro Leu Ser Arg Pro  
 2305 2310 2315 2320  
 Ile Gln Ser Pro Gly Arg Asn Ser Ile Ser Pro Gly Arg Asn Gly Ile  
 2325 2330 2335  
 Ser Pro Pro Asn Lys Leu Ser Gln Leu Pro Arg Thr Ser Ser Pro Ser  
 2340 2345 2350  
 Thr Ala Ser Thr Lys Ser Ser Gly Ser Gly Lys Met Ser Tyr Thr Ser  
 2355 2360 2365  
 Pro Gly Arg Gln Met Ser Gln Gln Asn Leu Thr Lys Gln Thr Gly Leu  
 2370 2375 2380  
 Ser Lys Asn Ala Ser Ser Ile Pro Arg Ser Glu Ser Ala Ser Lys Gly  
 2385 2390 2395 2400  
 Leu Asn Gln Met Asn Asn Gly Asn Gly Ala Asn Lys Lys Val Glu Leu  
 2405 2410 2415

Ser Arg Met Ser Ser Thr Lys Ser Ser Gly Ser Glu Ser Asp Arg Ser  
 2420 2425 2430  
 Glu Arg Pro Val Leu Val Arg Gln Ser Thr Phe Ile Lys Glu Ala Pro  
 2435 2440 2445  
 Ser Pro Thr Leu Arg Arg Lys Leu Glu Glu Ser Ala Ser Phe Glu Ser  
 2450 2455 2460  
 Leu Ser Pro Ser Ser Arg Pro Ala Ser Pro Thr Arg Ser Gln Ala Gln  
 2465 2470 2475 2480  
 Thr Pro Val Leu Ser Pro Ser Leu Pro Asp Met Ser Leu Ser Thr His  
 2485 2490 2495  
 Ser Ser Val Gln Ala Gly Gly Trp Arg Lys Leu Pro Pro Asn Leu Ser  
 2500 2505 2510  
 Pro Thr Ile Glu Tyr Asn Asp Gly Arg Pro Ala Lys Arg His Asp Ile  
 2515 2520 2525  
 Ala Arg Ser His Ser Glu Ser Pro Ser Arg Leu Pro Ile Asn Arg Ser  
 2530 2535 2540  
 Gly Thr Trp Lys Arg Glu His Ser Lys His Ser Ser Ser Leu Pro Arg  
 2545 2550 2555 2560  
 Val Ser Thr Trp Arg Arg Thr Gly Ser Ser Ser Ser Ile Leu Ser Ala  
 2565 2570 2575  
 Ser Ser Glu Ser Ser Glu Lys Ala Lys Ser Glu Asp Glu Lys His Val  
 2580 2585 2590  
 Asn Ser Ile Ser Gly Thr Lys Gln Ser Lys Glu Asn Gln Val Ser Ala  
 2595 2600 2605  
 Lys Gly Thr Trp Arg Lys Ile Lys Glu Asn Glu Phe Ser Pro Thr Asn  
 2610 2615 2620  
 Ser Thr Ser Gln Thr Val Ser Ser Gly Ala Thr Asn Gly Ala Glu Ser  
 2625 2630 2635 2640  
 Lys Thr Leu Ile Tyr Gln Met Ala Pro Ala Val Ser Lys Thr Glu Asp  
 2645 2650 2655  
 Val Trp Val Arg Ile Glu Asp Cys Pro Ile Asn Asn Pro Arg Ser Gly  
 2660 2665 2670  
 Arg Ser Pro Thr Gly Asn Thr Pro Pro Val Ile Asp Ser Val Ser Glu  
 2675 2680 2685  
 Lys Ala Asn Pro Asn Ile Lys Asp Ser Lys Asp Asn Gln Ala Lys Gln  
 2690 2695 2700  
 Asn Val Gly Asn Gly Ser Val Pro Met Arg Thr Val Gly Leu Glu Asn  
 2705 2710 2715 2720  
 Arg Leu Thr Ser Phe Ile Gln Val Asp Ala Pro Asp Gln Lys Gly Thr  
 2725 2730 2735  
 Glu Ile Lys Pro Gly Gln Asn Asn Pro Val Pro Val Ser Glu Thr Asn  
 2740 2745 2750

Glu Ser Pro Ile Val Glu Arg Thr Pro Phe Ser Ser Ser Ser Ser Ser  
 2755 2760 2765

Lys His Ser Ser Pro Ser Gly Thr Val Ala Ala Arg Val Thr Pro Phe  
 2770 2775 2780

Asn Tyr Asn Pro Ser Pro Arg Lys Ser Ser Ala Asp Ser Thr Ser Ala  
 2785 2790 2795 2800

Arg Pro Ser Gln Ile Pro Thr Pro Val Asn Asn Asn Thr Lys Lys Arg  
 2805 2810 2815

Asp Ser Lys Thr Asp Ser Thr Glu Ser Ser Gly Thr Gln Ser Pro Lys  
 2820 2825 2830

Arg His Ser Gly Ser Tyr Leu Val Thr Ser Val  
 2835 2840

## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3172 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: DP1(TB2)

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..630

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCA	GTC	GCC	GCT	CCA	GTC	TAT	CCG	GCA	CTA	GGA	ACA	GCC	CCG	GGN	GGC	48
Ala	Val	Ala	Ala	Pro	Val	Tyr	Pro	Ala	Leu	Gly	Thr	Ala	Pro	Gly	Gly	
1				5					10					15		
GAG	ACG	GTC	CCC	GCC	ATG	TCT	GCG	GCC	ATG	AGG	GAG	AGG	TTC	GAC	CGG	96
Glu	Thr	Val	Pro	Ala	Met	Ser	Ala	Ala	Met	Arg	Glu	Arg	Phe	Asp	Arg	
			20					25					30			
TTC	CTG	CAC	GAG	AAG	AAC	TGC	ATG	ACT	GAC	CTT	CTG	GCC	AAG	CTC	GAG	144
Phe	Leu	His	Glu	Lys	Asn	Cys	Met	Thr	Asp	Leu	Leu	Ala	Lys	Leu	Glu	
		35				40						45				
GCC	AAA	ACC	GCG	GTG	AAC	AGG	AGC	TTC	ATC	GCT	CTT	GGT	GTC	ATC	GGA	192
Ala	Lys	Thr	Gly	Val	Asn	Arg	Ser	Phe	Ile	Ala	Leu	Gly	Val	Ile	Gly	
		50				55					60					
CTC	GTG	GCC	TTG	TAC	CTG	GTG	TTC	GGT	TAT	GGA	GCC	TCT	CTC	CTC	TGC	240
Leu	Val	Ala	Leu	Tyr	Leu	Val	Phe	Gly	Tyr	Gly	Ala	Ser	Leu	Leu	Cys	
65					70					75					80	



AAC CTG ATA GGA TTT GGC TAC CCA GCC TAC ATC TCA ATT AAA GCT ATA	288
Asn Leu Ile Gly Phe Gly Tyr Pro Ala Tyr Ile Ser Ile Lys Ala Ile	
85 90 95	
GAG AGT CCC AAC AAA GAA GAT GAT ACC CAG TGG CTG ACC TAC TGG GTA	336
Glu Ser Pro Asn Lys Glu Asp Asp Thr Gln Trp Leu Thr Tyr Trp Val	
100 105 110	
GTG TAT GGT GTG TTC AGC ATT GCT GAA TTC TTC TCT GAT ATC TTC CTG	384
Val Tyr Gly Val Phe Ser Ile Ala Glu Phe Phe Ser Asp Ile Phe Leu	
115 120 125	
TCA TGG TTC CCC TTC TAC TAC ATG CTG AAG TGT GGC TTC CTG TTG TGG	432
Ser Trp Phe Pro Phe Tyr Tyr Met Leu Lys Cys Gly Phe Leu Leu Trp	
130 135 140	
TGC ATG GCC CCG AGC CCT TCT AAT GGG GCT GAA CTG CTC TAC AAG CGC	480
Cys Met Ala Pro Ser Pro Ser Asn Gly Ala Glu Leu Leu Tyr Lys Arg	
145 150 155 160	
ATC ATC CGT CCT TTC TTC CTG AAG CAC GAG TCC CAG ATG GAC AGT GTG	528
Ile Ile Arg Pro Phe Phe Leu Lys His Glu Ser Gln Met Asp Ser Val	
165 170 175	
GTC AAG GAC CTT AAA GAC AAG TCC AAA GAG ACT GCA GAT GCC ATC ACT	576
Val Lys Asp Leu Lys Asp Lys Ser Lys Glu Thr Ala Asp Ala Ile Thr	
180 185 190	
AAA GAA GCG AAG AAA GCT ACC GTG AAT TTA CTG GGT GAA GAA AAG AAG	624
Lys Glu Ala Lys Lys Ala Thr Val Asn Leu Leu Gly Glu Glu Lys Lys	
195 200 205	
AGC ACC TAAACCAGAC TAAACCAGAC TGGATGGAAA CTCCTGCC CTTCTGTACC	680
Ser Thr	
210	
TTCCTACTGG AGCTTGATGT TATATTAGGG ACTGTGGTAT AATTATTTTA ATAATGTTGC	740
CTTGGAACA TTTTGTAGAT ATTAAAGATT GGAATGTGTT GTAAGTTTCT TTGCTTACTT	800
TTACTGTCTA TATATATAGG GAGCACTTTA AACTTAATGC AGTGGCCAGT GTCCACGTTT	860
TTGGAAAATG TATTTTGCCT CTGGGTAGGA AAAGATGTAT GTTGCTATCC TGCAGGAAAT	920
ATAAACTTAA AATAAAATTA TATACCCAC AGGCTGTGTA CTTTACTGGG CTCTCCCTGC	980
ACGSATTTTC TCTGTAGTTA CATTAGGRT AATCTTTATG GTTCTACTTC CTRTAATGTA	1040
CAATTTTATA TAATTCNGRA ATGTTTTTAA TGTATTTGTG CACATGTACA TATGGAATG	1100
TTACTGTCTG ACTACANCA GCATCATGCT CATGGGGAGG GAGCAGGGGA AGGTTGTATG	1160
TGTCATTTAT AACTTCTGTA CAGTAAGACC ACCTGCCAAA AGCTGGAGGA ACCATTGTGC	1220
TGGTGTGGTC TACTAAATAA TACTTTAGGA AATACGTGAT TAATATGCAA GTGAACAAAG	1280
TGAGAAATGA AATCGAATGG AGATTGGCCT GGTGTTTCC GTAGTATATG GCATATGAAT	1340
ACCAGGATAG CTTTATAAAG CAGTTAGTTA GTTAGTTACT CACTCTAGTG ATAAATCGGG	1400
AAATTTACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAC ACACACACAG	1460

AGTACCCTGT	AACTCTCAAT	TCCCTGAAAA	ACTAGTAATA	CTGTCTTATC	TGCTATAAAC	1520
TTTACATATT	TGTCTATTGT	CAAGATGCTA	CANTGGAMNC	CATTTCGGT	TTTATCTTCA	1580
NAGSGGAGAN	ACATGTTGAT	TTAGTCTTCT	TTCCCAATCT	TCTTTTTTAA	MCCAGTTTNA	1640
GGMNCTTCTG	RAGATTTGYC	CACCTCTGAT	TACATGTATG	TTCTYGTTG	TATCATKAGC	1700
AACAACATGC	TAATGRCGAC	ACCTAGCTCT	RAGMGCAATT	CTGGGAGANT	GARAGGNWGT	1760
ATARAGTMNC	CCATAATCTG	CTTGGCAATA	GTAAAGTCAA	TCTATCTTCA	GTTTTTCTCT	1820
GGCCTTTAAG	GTCAAACACA	AGAGGCTTCC	CTAGTTTACA	AGTCAGAGTC	ACTTGTAGTC	1880
CATTTAAATG	CCCTCATCCG	TATTCTTTGT	GTTGATAAGC	TGCACAKGAC	TACATAGTAA	1940
GTACAGANCA	GTAAAGTTAA	NNCGGATGTC	TCCATTGATC	TGCCAANTCG	NTATAGAGAG	2000
CAATTTGTCT	GGACTAGAAA	ATCTGAGTTT	TACACCATAC	TGTTAAGAGT	CCTTTTGAAT	2060
TAAACTAGAC	TAAAACAAGT	GTATACTAA	ACTAACAAGA	TTAAATATCC	AGCCAGTACA	2120
GTATTTTTTA	AGGCAAATAA	AGATGATTAG	CTCACCTTGA	GNTAACAATC	AGGTAAGATC	2180
ATNACAATGT	CTCATGATGT	NAANAATATT	AAAGATATCA	ATACTAAGTG	ACAGTATCAC	2240
NNCTAATATA	ATATGGATCA	GAGCATTAT	TTTGGGGAGG	AAAACAGTGG	TGATTACCGG	2300
CATTTTATTA	AACTTAAAC	TTTGTAGAAA	GCAAACAAAA	TTGTTCTTGG	GAGAAAAATCA	2360
ACTTTTAGAT	TAAAAAAATT	TTAAGTAWCT	AGGAGTATTT	AAATCCTTTT	CCCATAAATA	2420
AAAGTACAGT	TTTCTTGGTG	GCAGAATGAA	AATCAGCAAC	NTCTAGCATA	TAGACTATAT	2480
AATCAGATTG	ACAGCATATA	GAATATATTA	TCAGACAAGA	TGAGGAGGTA	CAAAAGTTAC	2540
TATTGCTCAT	AATGACTTAC	AGGCTAAAA	TAGNTNTAAA	ATACTATATT	AAATTCTGAA	2600
TGCAATTTTT	TTTTGTTCCC	TTGAGACCAA	AATTTAAGTT	AACTGTTGCT	GGCAGTCTAA	2660
GTGTAAATGT	TAACAGCAGG	AGAAGTTAAG	AATTGAGCAG	TTCTGTTGCA	TGATTTCCCA	2720
AATGAAATAC	TGCCTTGGCT	AGAGTTTGAA	AACTAATTG	AGCCTGTGCC	TGGCTAGAAA	2780
ACAAGCGTTT	ATTTGAATGT	GAATAGTGTT	TCAAAGGTAT	GTAGTTACAG	AATTCCTACC	2840
AAACAGCTTA	AATTCTTCAA	GAAAGAATTC	CTGCAGCAGT	TATTCCCTTA	CCTGAAGGCT	2900
TCAATCATTT	GGATCAACAA	CTGCTACTCT	CGGGAAGACT	CCTCTACTCA	CAGCTGAAGA	2960
AAATGAGCAC	ACCCTTCACA	CTGTTATCAC	CTATCCTGAA	GATGTGATAC	ACTGAATGGA	3020
AATAAATAGA	TGTAAATAAA	ATTGAGWTCT	CATTTAAAA	AAACCATGTG	CCCAATGGGA	3080
AAATGACCTC	ATGTTGTGGT	TTAAACAGCA	ACTGCACCCA	CTAGCACAGC	CCATTGAGCT	3140
ANCCTATATA	TACATCTCTG	TCAGTGCCCC	TC			3172

(2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 210 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ala Val Ala Ala Pro Val Tyr Pro Ala Leu Gly Thr Ala Pro Gly Gly  
 1 5 10 15  
 Glu Thr Val Pro Ala Met Ser Ala Ala Met Arg Glu Arg Phe Asp Arg  
 20 25 30  
 Phe Leu His Glu Lys Asn Cys Met Thr Asp Leu Leu Ala Lys Leu Glu  
 35 40 45  
 Ala Lys Thr Gly Val Asn Arg Ser Phe Ile Ala Leu Gly Val Ile Gly  
 50 55 60  
 Leu Val Ala Leu Tyr Leu Val Phe Gly Tyr Gly Ala Ser Leu Leu Cys  
 65 70 75 80  
 Asn Leu Ile Gly Phe Gly Tyr Pro Ala Tyr Ile Ser Ile Lys Ala Ile  
 85 90 95  
 Glu Ser Pro Asn Lys Glu Asp Asp Thr Gln Trp Leu Thr Tyr Trp Val  
 100 105 110  
 Val Tyr Gly Val Phe Ser Ile Ala Glu Phe Phe Ser Asp Ile Phe Leu  
 115 120 125  
 Ser Trp Phe Pro Phe Tyr Tyr Met Leu Lys Cys Gly Phe Leu Leu Trp  
 130 135 140  
 Cys Met Ala Pro Ser Pro Ser Asn Gly Ala Glu Leu Leu Tyr Lys Arg  
 145 150 155 160  
 Ile Ile Arg Pro Phe Phe Leu Lys His Glu Ser Gln Met Asp Ser Val  
 165 170 175  
 Val Lys Asp Leu Lys Asp Lys Ser Lys Glu Thr Ala Asp Ala Ile Thr  
 180 185 190  
 Lys Glu Ala Lys Lys Ala Thr Val Asn Leu Leu Gly Glu Glu Lys Lys  
 195 200 205  
 Ser Thr  
 210

## (2) INFORMATION FOR SEQ ID NO:5:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 434 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

- (vi) ORIGINAL SOURCE:  
 (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: TB1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Val	Ala	Pro	Val	Val	Val	Gly	Ser	Gly	Arg	Ala	Pro	Arg	His	Pro	Ala	1	5	10	15
Pro	Ala	Ala	Met	His	Pro	Arg	Arg	Pro	Asp	Gly	Phe	Asp	Gly	Leu	Gly	20	25	30	
Tyr	Arg	Gly	Gly	Ala	Arg	Asp	Glu	Gln	Gly	Phe	Gly	Gly	Ala	Phe	Pro	35	40	45	
Ala	Arg	Ser	Phe	Ser	Thr	Gly	Ser	Asp	Leu	Gly	His	Trp	Val	Thr	Thr	50	55	60	
Pro	Pro	Asp	Ile	Pro	Gly	Ser	Arg	Asn	Leu	His	Trp	Gly	Glu	Lys	Ser	65	70	75	80
Pro	Pro	Tyr	Gly	Val	Pro	Thr	Thr	Ser	Thr	Pro	Tyr	Glu	Gly	Pro	Thr	85	90	95	
Glu	Glu	Pro	Phe	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Val	Gln	Gly	Gln	Ser	100	105	110	
Ser	Glu	Gln	Leu	Asn	Arg	Phe	Ala	Gly	Phe	Gly	Ile	Gly	Leu	Ala	Ser	115	120	125	
Leu	Phe	Thr	Glu	Asn	Val	Leu	Ala	His	Pro	Cys	Ile	Val	Leu	Arg	Arg	130	135	140	
Gln	Cys	Gln	Val	Asn	Tyr	His	Ala	Gln	His	Tyr	His	Leu	Thr	Pro	Phe	145	150	155	160
Thr	Val	Ile	Asn	Ile	Met	Tyr	Ser	Phe	Asn	Lys	Thr	Gln	Gly	Pro	Arg	165	170	175	
Ala	Leu	Trp	Lys	Gly	Met	Gly	Ser	Thr	Phe	Ile	Val	Gln	Gly	Val	Thr	180	185	190	
Leu	Gly	Ala	Glu	Gly	Ile	Ile	Ser	Glu	Phe	Thr	Pro	Leu	Pro	Arg	Glu	195	200	205	
Val	Leu	His	Lys	Trp	Ser	Pro	Lys	Gln	Ile	Gly	Glu	His	Leu	Leu	Leu	210	215	220	
Lys	Ser	Leu	Thr	Tyr	Val	Val	Ala	Met	Pro	Phe	Tyr	Ser	Ala	Ser	Leu	225	230	235	240
Ile	Glu	Thr	Val	Gln	Ser	Glu	Ile	Ile	Arg	Asp	Asn	Thr	Gly	Ile	Leu	245	250	255	
Glu	Cys	Val	Lys	Glu	Gly	Ile	Gly	Arg	Val	Ile	Gly	Met	Gly	Val	Pro	260	265	270	

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His Ser Lys Arg Leu Leu Pro Leu Leu Ser Leu Ile Phe Pro Thr Val  
 275 280 285  
 Leu His Gly Val Leu His Tyr Ile Ile Ser Ser Val Ile Gln Lys Phe  
 290 295 300  
 Val Leu Leu Ile Leu Lys Arg Lys Thr Tyr Asn Ser His Leu Ala Glu  
 305 310 315 320  
 Ser Thr Ser Pro Val Gln Ser Met Leu Asp Ala Tyr Phe Pro Glu Leu  
 325 330 335  
 Ile Ala Asn Phe Ala Ala Ser Leu Cys Ser Asp Val Ile Leu Tyr Pro  
 340 345 350  
 Leu Glu Thr Val Leu His Arg Leu His Ile Gln Gly Thr Arg Thr Ile  
 355 360 365  
 Ile Asp Asn Thr Asp Leu Gly Tyr Glu Val Leu Pro Ile Asn Thr Gln  
 370 375 380  
 Tyr Glu Gly Met Arg Asp Cys Ile Asn Thr Ile Arg Gln Glu Glu Gly  
 385 390 395 400  
 Val Phe Gly Phe Tyr Lys Gly Phe Gly Ala Val Ile Ile Gln Tyr Thr  
 405 410 415  
 Leu His Ala Ala Val Leu Gln Ile Thr Lys Ile Ile Tyr Ser Thr Leu  
 420 425 430  
 Leu Gln

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 185 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
- (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:
- (B) CLONE: YS-39(TB2)
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
- Glu Leu Arg Arg Phe Asp Arg Phe Leu His Glu Lys Asn Cys Met Thr  
 1 5 10 15  
 Asp Leu Leu Ala Lys Leu Glu Ala Lys Thr Gly Val Asn Arg Ser Phe  
 20 25 30  
 Ile Ala Leu Gly Val Ile Gly Leu Val Ala Leu Tyr Leu Val Phe Gly  
 35 40 45

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Tyr Gly Ala Ser Leu Leu Cys Asn Leu Ile Gly Phe Gly Tyr Pro Ala  
 50 55 60  
 Tyr Ile Ser Ile Lys Ala Ile Glu Ser Pro Asn Lys Glu Asp Asp Thr  
 65 70 75 80  
 Gln Trp Leu Thr Tyr Trp Val Val Tyr Gly Val Phe Ser Ile Ala Glu  
 85 90 95  
 Phe Phe Ser Asp Ile Phe Leu Ser Trp Phe Pro Phe Tyr Tyr Ile Leu  
 100 105 110  
 Lys Cys Gly Phe Leu Leu Trp Cys Met Ala Pro Ser Pro Ser Asn Gly  
 115 120 125  
 Ala Glu Leu Leu Tyr Lys Arg Ile Ile Arg Pro Phe Phe Leu Lys His  
 130 135 140  
 Glu Ser Gln Met Asp Ser Val Val Lys Asp Leu Lys Asp Lys Ala Lys  
 145 150 155 160  
 Glu Thr Ala Asp Ala Ile Thr Lys Glu Ala Lys Lys Ala Thr Val Asn  
 165 170 175  
 Leu Leu Gly Glu Glu Lys Lys Ser Thr  
 180 185

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 2842 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: APC
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
 

Met Ala Ala Ala Ser Tyr Asp Gln Leu Leu Lys Gln Val Glu Ala Leu  
 1 5 10 15  
 Lys Met Glu Asn Ser Asn Leu Arg Gln Glu Leu Glu Asp Asn Ser Asn  
 20 25 30  
 His Leu Thr Lys Leu Glu Thr Glu Ala Ser Asn Met Lys Glu Val Leu  
 35 40 45  
 Lys Gln Leu Gln Gly Ser Ile Glu Asp Glu Ala Met Ala Ser Ser Gly  
 50 55 60  
 Gln Ile Asp Leu Leu Glu Arg Leu Lys Glu Leu Asn Leu Asp Ser Ser  
 65 70 75 80

Asn Phe Pro Gly Val Lys Leu Arg Ser Lys Met Ser Leu Arg Ser Tyr  
 85 90 95  
 Gly Ser Arg Glu Gly Ser Val Ser Ser Arg Ser Gly Glu Cys Ser Pro  
 100 105 110  
 Val Pro Met Gly Ser Phe Pro Arg Arg Gly Phe Val Asn Gly Ser Arg  
 115 120 125  
 Glu Ser Thr Gly Tyr Leu Glu Glu Leu Glu Lys Glu Arg Ser Leu Leu  
 130 135 140  
 Leu Ala Asp Leu Asp Lys Glu Glu Lys Glu Lys Asp Trp Tyr Tyr Ala  
 145 150 155 160  
 Gln Leu Gln Asn Leu Thr Lys Arg Ile Asp Ser Leu Leu Thr Glu Asn  
 165 170 175  
 Phe Ser Leu Gln Thr Asp Met Thr Arg Arg Gln Leu Glu Tyr Glu Ala  
 180 185 190  
 Arg Gln Ile Arg Val Ala Met Glu Glu Gln Leu Gly Thr Cys Gln Asp  
 195 200 205  
 Met Glu Lys Arg Ala Gln Arg Arg Ile Ala Arg Ile Gln Gln Ile Glu  
 210 215 220  
 Lys Asp Ile Leu Arg Ile Arg Gln Leu Leu Gln Ser Gln Ala Thr Glu  
 225 230 235 240  
 Ala Glu Arg Ser Ser Gln Asn Lys His Glu Thr Gly Ser His Asp Ala  
 245 250 255  
 Glu Arg Gln Asn Glu Gly Gln Gly Val Gly Glu Ile Asn Met Ala Thr  
 260 265 270  
 Ser Gly Asn Gly Gln Gly Ser Thr Thr Arg Met Asp His Glu Thr Ala  
 275 280 285  
 Ser Val Leu Ser Ser Ser Ser Thr His Ser Ala Pro Arg Arg Leu Thr  
 290 295 300  
 Ser His Leu Gly Thr Lys Val Glu Met Val Tyr Ser Leu Leu Ser Met  
 305 310 315 320  
 Leu Gly Thr His Asp Lys Asp Asp Met Ser Arg Thr Leu Leu Ala Met  
 325 330 335  
 Ser Ser Ser Gln Asp Ser Cys Ile Ser Met Arg Gln Ser Gly Cys Leu  
 340 345 350  
 Pro Leu Leu Ile Gln Leu Leu His Gly Asn Asp Lys Asp Ser Val Leu  
 355 360 365  
 Leu Gly Asn Ser Arg Gly Ser Lys Glu Ala Arg Ala Arg Ala Ser Ala  
 370 375 380  
 Ala Leu His Asn Ile Ile His Ser Gln Pro Asp Asp Lys Arg Gly Arg  
 385 390 395 400  
 Arg Glu Ile Arg Val Leu His Leu Leu Glu Gln Ile Arg Ala Tyr Cys  
 405 410 415

Glu Thr Cys Trp Glu Trp Gln Glu Ala His Glu Pro Gly Met Asp Gln  
 420 425 430  
 Asp Lys Asn Pro Met Pro Ala Pro Val Glu His Gln Ile Cys Pro Ala  
 435 440 445  
 Val Cys Val Leu Met Lys Leu Ser Phe Asp Glu Glu His Arg His Ala  
 450 455 460  
 Met Asn Glu Leu Gly Gly Leu Gln Ala Ile Ala Glu Leu Leu Gln Val  
 465 470 475 480  
 Asp Cys Glu Met Tyr Gly Leu Thr Asn Asp His Tyr Ser Ile Thr Leu  
 485 490 495  
 Arg Arg Tyr Ala Gly Met Ala Leu Thr Asn Leu Thr Phe Gly Asp Val  
 500 505 510  
 Ala Asn Lys Ala Thr Leu Cys Ser Met Lys Gly Cys Met Arg Ala Leu  
 515 520 525  
 Val Ala Gln Leu Lys Ser Glu Ser Glu Asp Leu Gln Gln Val Ile Ala  
 530 535 540  
 Ser Val Leu Arg Asn Leu Ser Trp Arg Ala Asp Val Asn Ser Lys Lys  
 545 550 555 560  
 Thr Leu Arg Glu Val Gly Ser Val Lys Ala Leu Met Glu Cys Ala Leu  
 565 570 575  
 Glu Val Lys Lys Glu Ser Thr Leu Lys Ser Val Leu Ser Ala Leu Trp  
 580 585 590  
 Asn Leu Ser Ala His Cys Thr Glu Asn Lys Ala Asp Ile Cys Ala Val  
 595 600 605  
 Asp Gly Ala Leu Ala Phe Leu Val Gly Thr Leu Thr Tyr Arg Ser Gln  
 610 615 620  
 Thr Asn Thr Leu Ala Ile Ile Glu Ser Gly Gly Gly Ile Leu Arg Asn  
 625 630 635 640  
 Val Ser Ser Leu Ile Ala Thr Asn Glu Asp His Arg Gln Ile Leu Arg  
 645 650 655  
 Glu Asn Asn Cys Leu Gln Thr Leu Leu Gln His Leu Lys Ser His Ser  
 660 665 670  
 Leu Thr Ile Val Ser Asn Ala Cys Gly Thr Leu Trp Asn Leu Ser Ala  
 675 680 685  
 Arg Asn Pro Lys Asp Gln Glu Ala Leu Trp Asp Met Gly Ala Val Ser  
 690 695 700  
 Met Leu Lys Asn Leu Ile His Ser Lys His Lys Met Ile Ala Met Gly  
 705 710 715 720  
 Ser Ala Ala Ala Leu Arg Asn Leu Met Ala Asn Arg Pro Ala Lys Tyr  
 725 730 735  
 Lys Asp Ala Asn Ile Met Ser Pro Gly Ser Ser Leu Pro Ser Leu His  
 740 745 750



Val Arg Lys Gln Lys Ala Leu Glu Ala Glu Leu Asp Ala Gln His Leu  
 755 760 765  
 Ser Glu Thr Phe Asp Asn Ile Asp Asn Leu Ser Pro Lys Ala Ser His  
 770 775 780  
 Arg Ser Lys Gln Arg His Lys Gln Ser Leu Tyr Gly Asp Tyr Val Phe  
 785 790 795 800  
 Asp Thr Asn Arg His Asp Asp Asn Arg Ser Asp Asn Phe Asn Thr Gly  
 805 810 815  
 Asn Met Thr Val Leu Ser Pro Tyr Leu Asn Thr Thr Val Leu Pro Ser  
 820 825 830  
 Ser Ser Ser Ser Arg Gly Ser Leu Asp Ser Ser Arg Ser Glu Lys Asp  
 835 840 845  
 Arg Ser Leu Glu Arg Glu Arg Gly Ile Gly Leu Gly Asn Tyr His Pro  
 850 855 860  
 Ala Thr Glu Asn Pro Gly Thr Ser Ser Lys Arg Gly Leu Gln Ile Ser  
 865 870 875 880  
 Thr Thr Ala Ala Gln Ile Ala Lys Val Met Glu Glu Val Ser Ala Ile  
 885 890 895  
 His Thr Ser Gln Glu Asp Arg Ser Ser Gly Ser Thr Thr Glu Leu His  
 900 905 910  
 Cys Val Thr Asp Glu Arg Asn Ala Leu Arg Arg Ser Ser Ala Ala His  
 915 920 925  
 Thr His Ser Asn Thr Tyr Asn Phe Thr Lys Ser Glu Asn Ser Asn Arg  
 930 935 940  
 Thr Cys Ser Met Pro Tyr Ala Lys Leu Glu Tyr Lys Arg Ser Ser Asn  
 945 950 955 960  
 Asp Ser Leu Asn Ser Val Ser Ser Ser Asp Gly Tyr Gly Lys Arg Gly  
 965 970 975  
 Gln Met Lys Pro Ser Ile Glu Ser Tyr Ser Glu Asp Asp Glu Ser Lys  
 980 985 990  
 Phe Cys Ser Tyr Gly Gln Tyr Pro Ala Asp Leu Ala His Lys Ile His  
 995 1000 1005  
 Ser Ala Asn His Met Asp Asp Asn Asp Gly Glu Leu Asp Thr Pro Ile  
 1010 1015 1020  
 Asn Tyr Ser Leu Lys Tyr Ser Asp Glu Gln Leu Asn Ser Gly Arg Gln  
 1025 1030 1035 1040  
 Ser Pro Ser Gln Asn Glu Arg Trp Ala Arg Pro Lys His Ile Ile Glu  
 1045 1050 1055  
 Asp Glu Ile Lys Gln Ser Glu Gln Arg Gln Ser Arg Asn Gln Ser Thr  
 1060 1065 1070  
 Thr Tyr Pro Val Tyr Thr Glu Ser Thr Asp Asp Lys His Leu Lys Phe  
 1075 1080 1085

Gln Pro His Phe Gly Gln Gln Glu Cys Val Ser Pro Tyr Arg Ser Arg  
 1090 1095 1100  
 Gly Ala Asn Gly Ser Glu Thr Asn Arg Val Gly Ser Asn His Gly Ile  
 1105 1110 1115 1120  
 Asn Gln Asn Val Ser Gln Ser Leu Cys Gln Glu Asp Asp Tyr Glu Asp  
 1125 1130 1135  
 Asp Lys Pro Thr Asn Tyr Ser Glu Arg Tyr Ser Glu Glu Glu Gln His  
 1140 1145 1150  
 Glu Glu Glu Glu Arg Pro Thr Asn Tyr Ser Ile Lys Tyr Asn Glu Glu  
 1155 1160 1165  
 Lys Arg His Val Asp Gln Pro Ile Asp Tyr Ser Leu Lys Tyr Ala Thr  
 1170 1175 1180  
 Asp Ile Pro Ser Ser Gln Lys Gln Ser Phe Ser Phe Ser Lys Ser Ser  
 1185 1190 1195 1200  
 Ser Gly Gln Ser Ser Lys Thr Glu His Met Ser Ser Ser Ser Glu Asn  
 1205 1210 1215  
 Thr Ser Thr Pro Ser Ser Asn Ala Lys Arg Gln Asn Gln Leu His Pro  
 1220 1225 1230  
 Ser Ser Ala Gln Ser Arg Ser Gly Gln Pro Gln Lys Ala Ala Thr Cys  
 1235 1240 1245  
 Lys Val Ser Ser Ile Asn Gln Glu Thr Ile Gln Thr Tyr Cys Val Glu  
 1250 1255 1260  
 Asp Thr Pro Ile Cys Phe Ser Arg Cys Ser Ser Leu Ser Ser Leu Ser  
 1265 1270 1275 1280  
 Ser Ala Glu Asp Glu Ile Gly Cys Asn Gln Thr Thr Gln Glu Ala Asp  
 1285 1290 1295  
 Ser Ala Asn Thr Leu Gln Ile Ala Glu Ile Lys Glu Lys Ile Gly Thr  
 1300 1305 1310  
 Arg Ser Ala Glu Asp Pro Val Ser Glu Val Pro Ala Val Ser Gln His  
 1315 1320 1325  
 Pro Arg Thr Lys Ser Ser Arg Leu Gln Gly Ser Ser Leu Ser Ser Glu  
 1330 1335 1340  
 Ser Ala Arg His Lys Ala Val Glu Phe Ser Ser Gly Ala Lys Ser Pro  
 1345 1350 1355 1360  
 Ser Lys Ser Gly Ala Gln Thr Pro Lys Ser Pro Pro Glu His Tyr Val  
 1365 1370 1375  
 Gln Glu Thr Pro Leu Met Phe Ser Arg Cys Thr Ser Val Ser Ser Leu  
 1380 1385 1390  
 Asp Ser Phe Glu Ser Arg Ser Ile Ala Ser Ser Val Gln Ser Glu Pro  
 1395 1400 1405  
 Cys Ser Gly Met Val Ser Gly Ile Ile Ser Pro Ser Asp Leu Pro Asp  
 1410 1415 1420

Ser Pro Gly Gln Thr Met Pro Pro Ser Arg Ser Lys Thr Pro Pro Pro  
 1425 1430 1435 1440  
 Pro Pro Gln Thr Ala Gln Thr Lys Arg Glu Val Pro Lys Asn Lys Ala  
 1445 1450 1455  
 Pro Thr Ala Glu Lys Arg Glu Ser Gly Pro Lys Gln Ala Ala Val Asn  
 1460 1465 1470  
 Ala Ala Val Gln Arg Val Gln Val Leu Pro Asp Ala Asp Thr Leu Leu  
 1475 1480 1485  
 His Phe Ala Thr Glu Ser Thr Pro Asp Gly Phe Ser Cys Ser Ser Ser  
 1490 1495 1500  
 Leu Ser Ala Leu Ser Leu Asp Glu Pro Phe Ile Gln Lys Asp Val Glu  
 1505 1510 1515 1520  
 Leu Arg Ile Met Pro Pro Val Gln Glu Asn Asp Asn Gly Asn Glu Thr  
 1525 1530 1535  
 Glu Ser Glu Gln Pro Lys Glu Ser Asn Glu Asn Gln Glu Lys Glu Ala  
 1540 1545 1550  
 Glu Lys Thr Ile Asp Ser Glu Lys Asp Leu Leu Asp Asp Ser Asp Asp  
 1555 1560 1565  
 Asp Asp Ile Glu Ile Leu Glu Glu Cys Ile Ile Ser Ala Met Pro Thr  
 1570 1575 1580  
 Lys Ser Ser Arg Lys Ala Lys Lys Pro Ala Gln Thr Ala Ser Lys Leu  
 1585 1590 1595 1600  
 Pro Pro Pro Val Ala Arg Lys Pro Ser Gln Leu Pro Val Tyr Lys Leu  
 1605 1610 1615  
 Leu Pro Ser Gln Asn Arg Leu Gln Pro Gln Lys His Val Ser Phe Thr  
 1620 1625 1630  
 Pro Gly Asp Asp Met Pro Arg Val Tyr Cys Val Glu Gly Thr Pro Ile  
 1635 1640 1645  
 Asn Phe Ser Thr Ala Thr Ser Leu Ser Asp Leu Thr Ile Glu Ser Pro  
 1650 1655 1660  
 Pro Asn Glu Leu Ala Ala Gly Glu Gly Val Arg Gly Gly Ala Gln Ser  
 1665 1670 1675 1680  
 Gly Glu Phe Glu Lys Arg Asp Thr Ile Pro Thr Glu Gly Arg Ser Thr  
 1685 1690 1695  
 Asp Glu Ala Gln Gly Gly Lys Thr Ser Ser Val Thr Ile Pro Glu Leu  
 1700 1705 1710  
 Asp Asp Asn Lys Ala Glu Glu Gly Asp Ile Leu Ala Glu Cys Ile Asn  
 1715 1720 1725  
 Ser Ala Met Pro Lys Gly Lys Ser His Lys Pro Phe Arg Val Lys Lys  
 1730 1735 1740  
 Ile Met Asp Gln Val Gln Gln Ala Ser Ala Ser Ser Ser Ala Pro Asn  
 1745 1750 1755 1760

Lys Asn Gln Leu Asp Gly Lys Lys Lys Lys Pro Thr Ser Pro Val Lys  
 1765 1770 1775  
 Pro Ile Pro Gln Asn Thr Glu Tyr Arg Thr Arg Val Arg Lys Asn Ala  
 1780 1785 1790  
 Asp Ser Lys Asn Asn Leu Asn Ala Glu Arg Val Phe Ser Asp Asn Lys  
 1795 1800 1805  
 Asp Ser Lys Lys Gln Asn Leu Lys Asn Asn Ser Lys Asp Phe Asn Asp  
 1810 1815 1820  
 Lys Leu Pro Asn Asn Glu Asp Arg Val Arg Gly Ser Phe Ala Phe Asp  
 1825 1830 1835 1840  
 Ser Pro His His Tyr Thr Pro Ile Glu Gly Thr Pro Tyr Cys Phe Ser  
 1845 1850 1855  
 Arg Asn Asp Ser Leu Ser Ser Leu Asp Phe Asp Asp Asp Val Asp  
 1860 1865 1870  
 Leu Ser Arg Glu Lys Ala Glu Leu Arg Lys Ala Lys Glu Asn Lys Glu  
 1875 1880 1885  
 Ser Glu Ala Lys Val Thr Ser His Thr Glu Leu Thr Ser Asn Gln Gln  
 1890 1895 1900  
 Ser Ala Asn Lys Thr Gln Ala Ile Ala Lys Gln Pro Ile Asn Arg Gly  
 1905 1910 1915 1920  
 Gln Pro Lys Pro Ile Leu Gln Lys Gln Ser Thr Phe Pro Gln Ser Ser  
 1925 1930 1935  
 Lys Asp Ile Pro Asp Arg Gly Ala Ala Thr Asp Glu Lys Leu Gln Asn  
 1940 1945 1950  
 Phe Ala Ile Glu Asn Thr Pro Val Cys Phe Ser His Asn Ser Ser Leu  
 1955 1960 1965  
 Ser Ser Leu Ser Asp Ile Asp Gln Glu Asn Asn Asn Lys Glu Asn Glu  
 1970 1975 1980  
 Pro Ile Lys Glu Thr Glu Pro Pro Asp Ser Gln Gly Glu Pro Ser Lys  
 1985 1990 1995 2000  
 Pro Gln Ala Ser Gly Tyr Ala Pro Lys Ser Phe His Val Glu Asp Thr  
 2005 2010 2015  
 Pro Val Cys Phe Ser Arg Asn Ser Ser Leu Ser Ser Leu Ser Ile Asp  
 2020 2025 2030  
 Ser Glu Asp Asp Leu Leu Gln Glu Cys Ile Ser Ser Ala Met Pro Lys  
 2035 2040 2045  
 Lys Lys Lys Pro Ser Arg Leu Lys Gly Asp Asn Glu Lys His Ser Pro  
 2050 2055 2060  
 Arg Asn Met Gly Gly Ile Leu Gly Glu Asp Leu Thr Leu Asp Leu Lys  
 2065 2070 2075 2080  
 Asp Ile Gln Arg Pro Asp Ser Glu His Gly Leu Ser Pro Asp Ser Glu  
 2085 2090 2095

Asn Phe Asp Trp Lys Ala Ile Gln Glu Gly Ala Asn Ser Ile Val Ser  
 2100 2105 2110  
 Ser Leu His Gln Ala Ala Ala Ala Cys Leu Ser Arg Gln Ala Ser  
 2115 2120 2125  
 Ser Asp Ser Asp Ser Ile Leu Ser Leu Lys Ser Gly Ile Ser Leu Gly  
 2130 2135 2140  
 Ser Pro Phe His Leu Thr Pro Asp Gln Glu Glu Lys Pro Phe Thr Ser  
 2145 2150 2155 2160  
 Asn Lys Gly Pro Arg Ile Leu Lys Pro Gly Glu Lys Ser Thr Leu Glu  
 2165 2170 2175  
 Thr Lys Lys Ile Glu Ser Glu Ser Lys Gly Ile Lys Gly Gly Lys Lys  
 2180 2185 2190  
 Val Tyr Lys Ser Leu Ile Thr Gly Lys Val Arg Ser Asn Ser Glu Ile  
 2195 2200 2205  
 Ser Gly Gln Met Lys Gln Pro Leu Gln Ala Asn Met Pro Ser Ile Ser  
 2210 2215 2220  
 Arg Gly Arg Thr Met Ile His Ile Pro Gly Val Arg Asn Ser Ser Ser  
 2225 2230 2235 2240  
 Ser Thr Ser Pro Val Ser Lys Lys Gly Pro Pro Leu Lys Thr Pro Ala  
 2245 2250 2255  
 Ser Lys Ser Pro Ser Glu Gly Gln Thr Ala Thr Thr Ser Pro Arg Gly  
 2260 2265 2270  
 Ala Lys Pro Ser Val Lys Ser Glu Leu Ser Pro Val Ala Arg Gln Thr  
 2275 2280 2285  
 Ser Gln Ile Gly Gly Ser Ser Lys Ala Pro Ser Arg Ser Gly Ser Arg  
 2290 2295 2300  
 Asp Ser Thr Pro Ser Arg Pro Ala Gln Gln Pro Leu Ser Arg Pro Ile  
 2305 2310 2315 2320  
 Gln Ser Pro Gly Arg Asn Ser Ile Ser Pro Gly Arg Asn Gly Ile Ser  
 2325 2330 2335  
 Pro Pro Asn Lys Leu Ser Gln Leu Pro Arg Thr Ser Ser Pro Ser Thr  
 2340 2345 2350  
 Ala Ser Thr Lys Ser Ser Gly Ser Gly Lys Met Ser Tyr Thr Ser Pro  
 2355 2360 2365  
 Gly Arg Gln Met Ser Gln Gln Asn Leu Thr Lys Gln Thr Gly Leu Ser  
 2370 2375 2380  
 Lys Asn Ala Ser Ser Ile Pro Arg Ser Glu Ser Ala Ser Lys Gly Leu  
 2385 2390 2395 2400  
 Asn Gln Met Asn Asn Gly Asn Gly Ala Asn Lys Lys Val Glu Leu Ser  
 2405 2410 2415  
 Arg Met Ser Ser Thr Lys Ser Ser Gly Ser Glu Ser Asp Arg Ser Glu  
 2420 2425 2430

Arg Pro Val Leu Val Arg Gln Ser Thr Phe Ile Lys Glu Ala Pro Ser  
 2435 2440 2445  
 Pro Thr Leu Arg Arg Lys Leu Glu Glu Ser Ala Ser Phe Glu Ser Leu  
 2450 2455 2460  
 Ser Pro Ser Ser Arg Pro Ala Ser Pro Thr Arg Ser Gln Ala Gln Thr  
 2465 2470 2475 2480  
 Pro Val Leu Ser Pro Ser Leu Pro Asp Met Ser Leu Ser Thr His Ser  
 2485 2490 2495  
 Ser Val Gln Ala Gly Gly Trp Arg Lys Leu Pro Pro Asn Leu Ser Pro  
 2500 2505 2510  
 Thr Ile Glu Tyr Asn Asp Gly Arg Pro Ala Lys Arg His Asp Ile Ala  
 2515 2520 2525  
 Arg Ser His Ser Glu Ser Pro Ser Arg Leu Pro Ile Asn Arg Ser Gly  
 2530 2535 2540  
 Thr Trp Lys Arg Glu His Ser Lys His Ser Ser Ser Leu Pro Arg Val  
 2545 2550 2555 2560  
 Ser Thr Trp Arg Arg Thr Gly Ser Ser Ser Ser Ile Leu Ser Ala Ser  
 2565 2570 2575  
 Ser Glu Ser Ser Glu Lys Ala Lys Ser Glu Asp Glu Lys His Val Asn  
 2580 2585 2590  
 Ser Ile Ser Gly Thr Lys Gln Ser Lys Glu Asn Gln Val Ser Ala Lys  
 2595 2600 2605  
 Gly Thr Trp Arg Lys Ile Lys Glu Asn Glu Phe Ser Pro Thr Asn Ser  
 2610 2615 2620  
 Thr Ser Gln Thr Val Ser Ser Gly Ala Thr Asn Gly Ala Glu Ser Lys  
 2625 2630 2635 2640  
 Thr Leu Ile Tyr Gln Met Ala Pro Ala Val Ser Lys Thr Glu Asp Val  
 2645 2650 2655  
 Trp Val Arg Ile Glu Asp Cys Pro Ile Asn Asn Pro Arg Ser Gly Arg  
 2660 2665 2670  
 Ser Pro Thr Gly Asn Thr Pro Pro Val Ile Asp Ser Val Ser Glu Lys  
 2675 2680 2685  
 Ala Asn Pro Asn Ile Lys Asp Ser Lys Asp Asn Gln Ala Lys Gln Asn  
 2690 2695 2700  
 Val Gly Asn Gly Ser Val Pro Met Arg Thr Val Gly Leu Glu Asn Arg  
 2705 2710 2715 2720  
 Leu Asn Ser Phe Ile Gln Val Asp Ala Pro Asp Gln Lys Gly Thr Glu  
 2725 2730 2735  
 Ile Lys Pro Gly Gln Asn Asn Pro Val Pro Val Ser Glu Thr Asn Glu  
 2740 2745 2750  
 Ser Ser Ile Val Glu Arg Thr Pro Phe Ser Ser Ser Ser Ser Ser Lys  
 2755 2760 2765

His Ser Ser Pro Ser Gly Thr Val Ala Ala Arg Val Thr Pro Phe Asn  
 2770 2775 2780  
 Tyr Asn Pro Ser Pro Arg Lys Ser Ser Ala Asp Ser Thr Ser Ala Arg  
 2785 2790 2795 2800  
 Pro Ser Gln Ile Pro Thr Pro Val Asn Asn Asn Thr Lys Lys Arg Asp  
 2805 2810 2815  
 Ser Lys Thr Asp Ser Thr Glu Ser Ser Gly Thr Gln Ser Pro Lys Arg  
 2820 2825 2830  
 His Ser Gly Ser Tyr Leu Val Thr Ser Val  
 2835 2840

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: ral2(yeast)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Leu Thr Gly Ala Lys Gly Leu Gln Leu Arg Ala Leu Arg Arg Ile Ala  
 1 5 10 15  
 Arg Ile Glu Gln Gly Gly Thr Ala Ile Ser Pro Thr Ser Pro Leu  
 20 25 30

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: peptide

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

## (vii) IMMEDIATE SOURCE:

- (B) CLONE: m3(mAChR)

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Leu Tyr Trp Arg Ile Tyr Lys Glu Thr Glu Lys Arg Thr Lys Glu Leu  
 1 5 10 15

Ala Gly Leu Gln Ala Ser Gly Thr Glu Ala Glu Thr Glu  
20 25

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: MCC

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Leu Tyr Pro Asn Leu Ala Glu Glu Arg Ser Arg Trp Glu Lys Glu Leu  
1 5 10 15  
Ala Gly Leu Arg Glu Glu Asn Glu Ser Leu Thr Ala Met  
20 25

(2) INFORMATION FOR SEQ ID NO:11:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GTATCAAGAC TGTGACTTTT AATTGTAGTT TATCCATTTT

40

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens



- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
TTTAGAATTT CATGTTAATA TATTGTGTTT TTTTAAACAG 40
- (2) INFORMATION FOR SEQ ID NO:13:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:  
GTAGATTTTA AAAAGGTGTT TTAAATAAT TTTTAAAGCT 40
- (2) INFORMATION FOR SEQ ID NO:14:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:  
AAGCAATTGT TGTATAAAAA CTTGTTTCTA TTTTATTAG 40
- (2) INFORMATION FOR SEQ ID NO:15:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:  
GTAACCTTTC TTCATATAGT AACATTGCC TTGTGTACTC 40

## (2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

NNNNNNNNNN NNNGTCCCTT TTTTAAAAA AAAAAAATAG

40

## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTAAGTAACT TGCGAGTACA ACTTATTGA AACTTTAATA

40

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ATACAAGATA TTGATACTTT TTTATTATTT GTGGTTTATG

40

## (2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 40 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GTAAGTTACT TGTTTCTAAG TGATAAAACA GYGAAGAGCT

40

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AATAAAAACA TAACTAATTA GGTTCCTTGT TTTATTTTAG

40

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GTTAGTAAAT TSCCTTTTTT GTTTGTGGGT ATAAAAATAG

40

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:  
ACCATTTTGT CATGTACTGA TGTAACTCC ATCTTAACAG 40

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:  
GTAAATAAAT TATTTTATCA TATTTTTTAA AATTATTTAA 40

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 64 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:  
CATGATGTTA TCTGTATTTA CCTATAGTCT AAATTATACC ATCTATAATG TGCTTAATTT 60  
TTAG 64

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 52 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:  
GTAACAGAAG ATTACAAACC CTGGTCACTA ATGCCATGAC TACTTTGCTA AG 52

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 46 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GGATATTAAA GTCGTAATTT TGTTTCTAAA CTCATTGGC CCACAG

46

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTATGTTCTC TATAGTGTAC ATCGTAGTGC ATGTTTCAA

40

## (2) INFORMATION FOR SEQ ID NO:28:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 56 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

CATCATGCT CTTCAAATAA CAAAGCATTG TGGTTTATGT TGATTTTATT TTTCAG

56

## (2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 43 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTAAGACAAA AATGTTTTTT AATGACATAG ACAATTACTG GTG

43

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TTAGATGATT GTCTTTTCC TCTGCCCTT TTAAATTAG

40

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 44 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

GTATGTTTT ATAACATGTA TTTCTTAAGA TAGCTCAGGT ATGA

44

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 54 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

GCTTGCTTC AAGTTGNCTT TTTAATGATC CTCTATTCTG TATTTAATTT ACAG

54

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 65 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GTACTATTTA GAATTTACAC TGTTTTCTT TTTTCTCTT TTCTTTGAGG CAGGGTCTCA  
CTCTG

60

65

(2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 52 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCAACTAGTA TGATTTTATG TATAAATTAA TCTAAAATTG ATTAATTTC AG

52

(2) INFORMATION FOR SEQ ID NO:35:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 42 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GTACCTTTGA AAACATTTAG TACTATAATA TGAATTTTAT GT

42

## (2) INFORMATION FOR SEQ ID NO:36:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 40 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

CCAACTCNA A TTAGATGACC CATATTCAGA AACTTACTAG

40

## (2) INFORMATION FOR SEQ ID NO:37:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 54 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GTATATATAG AGTTTTATAT TACTTTTAAA GTACAGAATT CATACTCTCA AAAA

54

## (2) INFORMATION FOR SEQ ID NO:38:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 41 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ATTGTGACCT TAATTTTGTG ATCTCTTGAT TTTTATTTC A G

41

## (2) INFORMATION FOR SEQ ID NO:39:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 18 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear



- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

TCCCCGCCTG CCGCTCTC

18

(2) INFORMATION FOR SEQ ID NO:40:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 18 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GCAGCGGCGG CTCCCGTG

18

(2) INFORMATION FOR SEQ ID NO:41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

GTGAACGGCT CTCATGCTGC

20

(2) INFORMATION FOR SEQ ID NO:42:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 19 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:  
ACGTGCGGGG AGGAATGGA 19
- (2) INFORMATION FOR SEQ ID NO:43:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:  
ATGATATCTT ACCAAATGAT ATAC 24
- (2) INFORMATION FOR SEQ ID NO:44:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:  
TTATTCTAC TTCTTCTATA CAG 23
- (2) INFORMATION FOR SEQ ID NO:45:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 21 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:  
TACCCATGCT GGCTCTTTT C 21

## (2) INFORMATION FOR SEQ ID NO:46:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

TGGGGCCATC TTGTTCTGA

20

## (2) INFORMATION FOR SEQ ID NO:47:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

ACATTAGGCA CAAAGCTTGC AA

22

## (2) INFORMATION FOR SEQ ID NO:48:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ATCAAGCTCC AGTAAGAAGG TA

22

## (2) INFORMATION FOR SEQ ID NO:49:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 19 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

TGCGGCTCCT GGGTTGTTG

19

(2) INFORMATION FOR SEQ ID NO:50:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

GCCCCTTCCT TTCTGAGGAC

20

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

TTTTCTCCTG CCTCTTACTG C

21

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

ATGACACCCC CCATTCCCTC

20

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

CCACTTAAAG CACATATATT TAGT

24

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

GTATGGAAAA TAGTGAAGAA CC

22

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

TTCTTAAGTC CTGTTTTTCT TTTC

24

## (2) INFORMATION FOR SEQ ID NO:56:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

TTTAGAACCT TTTTGTGTT GTG

23

## (2) INFORMATION FOR SEQ ID NO:57:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

CTCAGATTAT AACTAAGCC TAAC

24

## (2) INFORMATION FOR SEQ ID NO:58:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

CATGTCTCTT ACAGTAGTAC CA

22

## (2) INFORMATION FOR SEQ ID NO:59:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 20 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

AGGTCCAAGG GTAGCCAAGG

20

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TAAAAATGGA TAAACTACAA TAAAAG

27

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

AAATACAGAA TCATGTCTTG AAGT

24

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

ACACCTAAAG ATGACAATTT GAG

23

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

TAACTTAGAT AGCAGTAATT TCCC

24

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

ACAATAAACT GGAGTACACA AGG

23

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

ATAGGTCATT GCTTCTTGCT GAT

23



## (2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

TGAATTTTAA TGGATTACCT AGGT

24

## (2) INFORMATION FOR SEQ ID NO:67:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CTTTTTTTGC TTTTACTGAT TAACG

25

## (2) INFORMATION FOR SEQ ID NO:68:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 27 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TGTAATTCAT TTTATTCCTA ATAGCTC

27

## (2) INFORMATION FOR SEQ ID NO:69:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

GGTAGCCATA GTATGATTAT TTCT

24

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CTACCTATTT TTATACCCAC AAAC

24

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

AAGAAAGCCT ACACCATTTT TGC

23

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:  
GATCATTCTT AGAACCATCT TGC 23
- (2) INFORMATION FOR SEQ ID NO:73:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:  
ACCTATAGTC TAAATTATAC CATC 24
- (2) INFORMATION FOR SEQ ID NO:74:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 20 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:  
GTCATGGCAT TAGTGACCAG 20
- (2) INFORMATION FOR SEQ ID NO:75:
- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:  
AGTCGTAATT TTGTTTCTAA ACTC 24

## (2) INFORMATION FOR SEQ ID NO:76:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

TGAAGGACTC GGATTTCACG C

21

## (2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

TCATTCACTC ACAGCCTGAT GAC

23

## (2) INFORMATION FOR SEQ ID NO:78:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

GCTTTGAAAC ATGCACTACG AT

22

## (2) INFORMATION FOR SEQ ID NO:79:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

AAACATCATT GCTCTTCAAA TAAC

24

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

TACCATGATT TAAAAATCCA CCAG

24

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GATGATTGTC TTTTCCTCT TGC

23

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CTGAGCTATC TTAAGAAATA CATG

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 25 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

TTTTAAATGA TCCTCTATTC TGTAT

(2) INFORMATION FOR SEQ ID NO:84:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 24 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

ACAGAGTCAG ACCCTGCCTC AAAG

(2) INFORMATION FOR SEQ ID NO:85:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

TTTCTATTCT TACTGCTAGC ATT

## (2) INFORMATION FOR SEQ ID NO:86:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

ATACACAGGT AAGAAATTAG GA

22

## (2) INFORMATION FOR SEQ ID NO:87:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

TAGATGACCC ATATTCTGTT TC

22

## (2) INFORMATION FOR SEQ ID NO:88:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CAATTAGGTC TTTTTCAGAG TA

22

## (2) INFORMATION FOR SEQ ID NO:89:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: cDNA
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

GTTACTGCAT ACACATTGTG AC

22

(2) INFORMATION FOR SEQ ID NO:90:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

GCTTTTGTGTT TCCTAACATG AAG

23

(2) INFORMATION FOR SEQ ID NO:91:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

TCTCCACAG GTAATACTCC C

21

(2) INFORMATION FOR SEQ ID NO:92:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 21 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Homo sapiens



## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

GCTAGAACTG AATGGGGTAC G

21

## (2) INFORMATION FOR SEQ ID NO:93:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

CAGGACAAAA TAATCCTGTC CC

22

## (2) INFORMATION FOR SEQ ID NO:94:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

ATTTTCTTAG TTTCATTCTT CCTC

24

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International Application No: PCT/

/

<b>MICROORGANISMS</b>	
Optional Sheet in connection with the microorganism referred to on page <u>22</u> , line <u>23</u> of the description	
<b>A. IDENTIFICATION OF DEPOSIT</b> Further deposits are identified on an additional sheet <input type="checkbox"/>	
Name of depository institution: <b>NATIONAL COLLECTION OF INDUSTRIAL AND MARINE BACTERIA (NCIMB)</b>	
Address of depository institution (including postal code and country): <b>23 St. Machar Drive Aberdeen AB2 1RY, Scotland United Kingdom</b>	
Date of deposit: <b>17 December 1990</b>	Accession Number: <b>NCIMB 40353</b>
<b>B. ADDITIONAL INDICATIONS</b> (leave blank if not applicable). This information is continued on a separate attached sheet <input type="checkbox"/>	
<b>Saccharomyces cerevisiae SC/37HG4</b>	
<b>C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE</b> (If the indications are not for all designated States)	
<b>D. SEPARATE FURNISHING OF INDICATIONS</b> (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (Specify the general nature of the indications e.g. - Accession Number of Deposit)	
<b>E.</b> <input type="checkbox"/> This sheet was received with the international application when filed (to be checked by the receiving Office)	
_____ (Authorized Officer)	
<input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau is	
_____ (Authorized Officer)	

**CLAIMS**

1. A method of diagnosing or prognosing a neoplastic tissue of a human, comprising:

detecting somatic alteration of wild-type APC gene coding sequences or their expression products in a tumor tissue isolated from a human, said alteration indicating neoplasia of the tissue.

2. The method of claim 1 wherein the expression products are mRNA molecules.

3. The method of claim 2 wherein the alteration of wild-type APC mRNA is detected by hybridization of mRNA from said tissue to an APC gene probe.

4. The method of claim 1 wherein alteration of wild-type APC gene coding sequences is detected by observing shifts in electrophoretic mobility of single-stranded DNA on non-denaturing polyacrylamide gels.

5. The method of claim 1 wherein alteration of wild-type APC gene coding sequences is detected by hybridization of an APC gene coding sequence probe to genomic DNA isolated from said tissue.

6. The method of claim 5 further comprising:  
subjecting genomic DNA isolated from a non-neoplastic tissue of the human to Southern hybridization with the APC gene coding sequence probe; and  
comparing the hybridizations of the APC gene probe to said tumor and non-neoplastic tissues.

7. The method of claim 5 wherein the APC gene probe detects a restriction fragment length polymorphism.

8. The method of claim 1 wherein the alteration of wild-type APC gene coding sequences is detected by determining the sequence of all or part of an APC gene in said tissue using a polymerase chain reaction, deviations in the APC sequence determined from that of the sequence shown in Figure 7 (SEQ ID NO.: 1) suggesting neoplasia.

9. The method of claim 1 wherein the alteration of wild-type APC gene coding sequences is detected by identifying a mismatch between molecules (1) an APC gene or APC mRNA isolated from said tissue and (2) a nucleic acid probe complementary to the human wild-

type APC gene coding sequence, when molecules (1) and (2) are hybridized to each other to form a duplex.

10. The method of claim 5 wherein the APC gene probe hybridizes to an exon selected from the group consisting of: (1) nucleotides 822 to 930; and (2) nucleotides 931 to 1309; (3) nucleotides 1406 to 1545; and (4) nucleotides 1956 to 2256.

11. The method of claim 1 wherein the alteration of wild-type APC gene coding sequences is detected by amplification of APC gene sequences in said tissue and hybridization of the amplified APC sequences to nucleic acid probes which comprise APC sequences.

12. The method of claim 1 wherein the alteration of wild-type APC gene coding sequences is detected by molecular cloning of the APC genes in said tissue and sequencing all or part of the cloned APC gene.

13. The method of claim 1 wherein the detection of alteration of wild-type APC gene coding sequences comprises screening for a deletion mutation.

14. The method of claim 1 wherein the detection of alteration of wild-type APC gene coding sequences comprises screening for a point mutation.

15. The method of claim 1 wherein the detection of alteration of wild-type APC gene coding sequences comprises screening for an insertion mutation.

16. The method of claim 1 wherein the tumor tissue is a colorectal tissue.

17. The method of claim 6 wherein the non-neoplastic tissue isolated from a human is from colonic mucosa.

18. The method of claim 1 wherein the expression products are protein molecules.

19. The method of claim 18 wherein the alteration of wild-type APC protein is detected by immunoblotting.

20. The method of claim 18 wherein the alteration of wild-type APC protein is detected by immunocytochemistry.

21. The method of claim 18 wherein the alteration of wild-type APC protein is detected by assaying for binding interactions between APC protein of said tumor tissue and a second cellular protein.

22. The method of claim 21 wherein the second cellular protein is selected from the group consisting of MCC protein, wild-type APC protein, and a G protein.

23. The method of claim 18 wherein the alteration of wild-type APC protein is detected by assaying for phospholipid metabolites.

24. A method of supplying wild-type APC gene function to a cell which has lost said function by virtue of a mutation in an APC gene, comprising:

introducing a wild-type APC gene into a cell which has lost said gene function such that said wild-type APC gene is expressed in the cell.

25. The method of claim 24 wherein the wild-type APC gene introduced recombines with the endogenous mutant APC gene present in the cell by a double recombination event to correct the APC gene mutation.

26. A method of supplying wild-type APC gene function to a cell which has altered APC function by virtue of a mutation in an APC gene, comprising:

introducing a portion of a wild-type APC gene into a cell which has lost said gene function such that said portion is expressed in the cell, said portion encoding a part of the APC protein which is required for non-neoplastic growth of said cell.

27. A method of supplying wild-type APC gene function to a cell which has altered APC function by virtue of a mutation in an APC gene, comprising:

applying human wild-type APC protein to a cell which has lost wild-type APC function.

28. A method of supplying wild-type APC gene function to a cell which has altered APC gene function by virtue of a mutation in an APC gene, comprising:

introducing into the cell a molecule which mimics the function of wild-type APC protein.

29. A pair of single stranded DNA primers for determination of a nucleotide sequence of an APC gene by polymerase chain reaction, the sequence of said primers being derived from chromosome 5q band 21, wherein the use of said primers in a polymerase chain reaction results in synthesis of DNA having all or part of the sequence shown in Figure 7.

30. The primers of claim 29 which have restriction enzyme sites at each 5' end.

31. The pair of primers of claim 29 having sequences corresponding to APC introns.

32. A nucleic acid probe complementary to human wild-type APC gene coding sequences.

33. The nucleic acid probe of claim 31 which hybridizes to an exon selected from the group consisting of: (1) nucleotides 822 to 930; and (2) nucleotides 931 to 1309; (3) nucleotides 1406 to 1545; (4) nucleotides 1956 to 2256.

34. A kit for detecting alteration of wild-type APC genes comprising a battery of nucleic acid probes which in the aggregate hybridize to all nucleotides of the APC gene coding sequences.

35. A method of detecting the presence of a neoplastic tissue in a human, comprising:

detecting in a body sample isolated from a human alteration of a wild-type APC gene coding sequence or wild-type APC expression product, said alteration indicating the presence of a neoplastic tissue in the human.

36. The method of claim 35 wherein said body sample is selected from the group consisting of serum, stool, urine and sputum.

37. A method of detecting genetic predisposition to cancer, including familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS), in a human comprising:

detecting a germline alteration of wild-type APC gene coding sequences or their expression products in a human sample

selected from the group consisting of blood and fetal tissue, said alteration indicating predisposition to cancer.

38. The method of claim 37 wherein the expression products are mRNA molecules.

39. The method of claim 38 wherein the alteration of wild-type APC mRNA is detected by hybridization of mRNA from said tissue to an APC gene probe.

40. The method of claim 37 wherein alteration of wild-type APC gene coding sequences is detected by observing shifts in electrophoretic mobility of single-stranded DNA on non-denaturing polyacrylamide gels.

41. The method of claim 37 wherein alteration of wild-type APC gene coding sequences is detected by hybridization of an APC gene coding sequence probe to genomic DNA isolated from said tissue.

42. The method of claim 41 wherein the APC gene coding sequence probe detects a restriction fragment length polymorphism.

43. The method of claim 37 wherein the alteration of wild-type APC gene coding sequences is detected by determining the sequence of all or part of an APC gene in said tissue using a polymerase chain reaction, deviations in the APC sequence determined from the sequence of Figure 7 suggesting predisposition to cancer.

44. The method of claim 37 wherein the alteration of wild-type APC gene coding sequences is detected by identifying a mismatch between molecules (1) an APC gene or APC mRNA isolated from said tissue and (2) a nucleic acid probe complementary to the human wild-type APC gene coding sequence, when molecules (1) and (2) are hybridized to each other to form a duplex.

45. The method of claim 41 wherein the APC gene probe hybridizes to an exon selected from the group consisting of: (1) nucleotides 822 to 930; and (2) nucleotides 931 to 1309; (3) nucleotides 1406 to 1545 and (4) nucleotides 1956 to 2256.

46. The method of claim 37 wherein the alteration of wild-type APC gene coding sequences is detected by amplification of APC gene sequences in said tissue and hybridization of the amplified APC

sequences to nucleic acid probes which comprise APC gene coding sequences.

47. The method of claim 37 wherein the alteration of wild-type APC gene coding sequences is detected by molecular cloning of the APC genes in said tissue and sequencing all or part of the cloned APC gene.

48. The method of claim 37 wherein the detection of alteration of wild-type APC gene coding sequences comprises screening for a deletion mutation.

49. The method of claim 37 wherein the detection of alteration of wild-type APC gene coding sequences comprises screening for a point mutation.

50. The method of claim 37 wherein the detection of alteration of wild-type APC gene coding sequences comprises screening for an insertion mutation.

51. The method of claim 37 wherein the expression products are protein molecules.

52. The method of claim 51 wherein the alteration of wild-type APC protein is detected by immunoblotting.

53. The method of claim 51 wherein the alteration of wild-type APC protein is detected by immunocytochemistry.

54. The method of claim 51 wherein the alteration of wild-type APC protein is detected by assaying for binding interactions between APC protein isolated from said tissue and a second cellular protein.

55. The method of claim 54 wherein the second cellular protein is selected from the group consisting of MCC protein, wild-type APC protein and a G protein.

56. A method of screening for genetic predisposition to cancer, including familial adenomatous polyposis (FAP) and Gardner's Syndrome (GS), in a human comprising:

detecting among kindred persons the presence of a DNA polymorphism which is linked to a mutant APC allele in an individual having a genetic predisposition to cancer, said kindred being



genetically related to the individual, the presence of said polymorphism suggesting a predisposition to cancer.

57. A preparation of the human APC protein substantially free of other human proteins, the amino acid sequence of said protein corresponding to that shown in Figure 3 or 7 (SEQ ID NO: 1).

58. A preparation of antibodies immunoreactive with a human APC protein and not substantially immunoreactive with other human proteins.

59. A method of testing therapeutic agents for the ability to suppress a neoplastically transformed phenotype, comprising:

applying a test substance to a cultured epithelial cell which carries a mutation in an APC allele;

determining whether said test substance suppresses the neoplastically transformed phenotype of the cell.

60. The method of claim 59 wherein the cultured epithelial cell has been genetically engineered to carry the mutation in the APC allele.

61. A method of testing therapeutic agents for the ability to suppress neoplastic growth, comprising:

administering a test substance to an animal which carries a mutant APC allele in its genome;

determining whether said test substance prevents or suppresses the growth of tumors.

62. A transgenic animal which carries a mutant APC allele from a second animal species in its genome.

63. An animal which has been genetically engineered to contain an insertion mutation which disrupts an APC allele in its genome.

64. A cDNA molecule which encodes a protein having the amino acid sequence shown in Figure 3 or 7 (SEQ ID NO: 7 or 1).

65. An isolated DNA molecule which encodes a protein having the amino acid sequence shown in Figure 3 or 7 (SEQ ID NO: 7 or 1).

66. A yeast artificial chromosome which is known as 37HG4.

## APC EXONS

[illegible]

'Relative to predicted translation initiation site

**Small case letters represent introns, large case letters represent exons**

The entire 3' end of the cloned APC cDNA (nt 1956-8973) appeared to be encoded in this exon, as indicated by restriction endonuclease mapping and sequencing of cloned genomic DNA. The ORF ended at nt 8935. The extreme 3' end of the APC transcript has not yet been identified.

**TABLE IIA**  
Germline mutations of the APC gene in FAP and GS Patients

<u>EXTRA-COLONIC PATIENT DISEASE</u>	<u>CODON</u>	<u>NUCLEOTIDE CHANGE</u>	<u>AMINO CHANGE</u>	<u>AGE</u>	<u>ACID</u>
93 Osteoma	279	TCA-→T <u>G</u> A	Ser-→Stop	39	Mandibular
24	301	CGA-→T <u>G</u> A	Arg-→Stop	46	None
34 Tumor	301	CGA-→T <u>G</u> A	Arg-→Stop	27	Desmoid
21 Osteoma	413	CGC-→T <u>G</u> C	Arg-→Cys	24	Mandibular
60 Osteoma	712	TCA-→T <u>G</u> A	Ser-→Stop	37	Mandibular
3746	243	CAGAG-→CAG	splice-junction		
3460	301	CGA-→T <u>G</u> A	Arg-→Stop		
3827	456	CTTTCA-→CTTCA	frameshift		
3712	500	T-→ <u>G</u>	Tyr-→Stop		

\* The mutated nucleotides are underlined.

**SUBSTITUTE SHEET**

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TABLE III

## Somatic Mutations in Sporadic CRC Patients

<u>PATIENT</u>	<u>CODON<sup>1</sup></u>	<u>NUCLEOTIDE CHANGE</u>	<u>AMINO ACID CHANGE</u>
T35	MCC 12	GAG/ <u>gt</u> aga-> GAG/ <u>gt</u> aga	(Splice Donor)
T16	MCC 145	<u>ct</u> cag/GGA-> <u>gt</u> cag/GGA	(Splice Acceptor)
T47	MCC 267	CGG-> <u>CT</u> G	Arg->Leu
T81	MCC 490	TCG-> <u>T</u> IG	Ser->Leu
T35	MCC 506	CGG-> <u>C</u> AG	Arg->Gln
T91	MCC 698	GCT-> <u>G</u> IT	Ala->Val
T34	APC 288	CCAGT-> <u>CCCAGCC</u> AGT	(Insertion)
T27	APC 331	CGA-> <u>I</u> GA	Arg->Stop
T135	APC 437	CAA/ <u>gt</u> aa->CAA/ <u>gt</u> aa	(Splice Donor)
T201	APC 1338	CAG-> <u>I</u> AG	Gln->Stop

For splice site mutations, the codon nearest to the mutation is listed

The underlined nucleotides were mutant; small case letters represent introns, large case letters represent exons

TABLE III

## Sequences of Primers Used for SSCP Analysis

SP1		
Exon	Primer 1	Primer 2
1	UP-TCCTCCCTCTCTCTCTCTCTC	RP-CCAGCCGCGGCTCCCTT
2	UP-GTCAACCCCTCTCATCTCTC	RP-ACCTCCGCGGAGCAATGGA
3	UP-ATCATATCTTTACCAATCATATAC	RP-CTATTCTACTTCTTCTATACAG
4	UP-TACCCA TCTCTCTCTCTCTTTC	RP-TCCGCGCATCTTCTTCTCTTA
5	UP-ACATTACCCACAAAGCTTCA	RP-ATCAAGCTTCACTAAGAAAGTA
SP19		
1	UP-TCCGCTCTCTCTCTCTCTTTC	RP-CCCTCTCTCTCTCTCTCTCTC
2	UP-TTCTCTCTCTCTCTCTCTCTC	RP-ATCAACCCGCGGCTCTCTCTC
3	UP-CCACTTAAGCACAATATCTCTCT	RP-CTCTCAAAATATCTCTCTCTCTC
4	UP-TTCTTAAGCTCTCTCTCTCTCTTTC	RP-TTCAAAAGCTTTTCTCTCTCTTTC
5	UP-CTCAGATTATACACTAAGCTCTAC	RP-CACTCTCTCTCTCTCTCTCTCTC
SP2.3		
1	UP-AGCTCCAGGCTCTCTCTCTCTC	RP-TAAATATCACTAAGCTCTCTCTCTC
2	UP-AAATACCAATCTCTCTCTCTCTCT	RP-ACCTTAAGCTCTCTCTCTCTCTCTC
3	UP-TAAGCTCACTCTCTCTCTCTCTCTC	RP-ACATTAAGCTCTCTCTCTCTCTCTC
4	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-TAAATATCACTAAGCTCTCTCTCTC
5	UP-CTTCTCTCTCTCTCTCTCTCTCTCTC	RP-TCTCTCTCTCTCTCTCTCTCTCTCTC
6	UP-CTTCTCTCTCTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
7	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
8	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
9	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
10	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
11	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
12	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
13	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
14	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
15	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
16	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
17	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
18	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
19	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
20	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
21	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
22	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
23	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
24	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
25	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
26	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
27	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
28	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
29	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
30	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
31	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
32	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
33	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
34	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
35	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
36	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
37	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
38	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
39	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
40	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
41	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
42	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
43	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
44	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
45	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
46	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
47	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
48	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
49	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
50	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
51	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
52	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
53	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
54	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
55	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
56	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
57	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
58	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
59	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
60	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
61	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
62	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
63	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
64	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
65	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
66	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
67	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
68	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
69	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
70	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
71	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
72	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
73	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
74	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
75	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
76	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
77	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
78	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
79	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
80	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
81	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
82	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
83	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
84	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
85	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
86	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
87	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
88	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
89	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
90	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
91	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
92	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
93	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
94	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
95	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
96	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
97	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
98	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
99	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC
100	UP-ATCACTCACTCTCTCTCTCTCTCTC	RP-CTCTCTCTCTCTCTCTCTCTCTCTC

All primers are read in the 5' to 3' direction. The first primer in each pair lies 5' of the exon it amplifies; the second primer lies 3' of the exon it amplifies. Primers that lie within the exon are identified by an asterisk. UP represents the -21M13 universal primer sequence; RP represents the M13 reverse primer sequence.

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TABLE IV

Seven Different Versions of the 20-Amino Acid Repeat																				
Consensus: F • V E • T P • C F S R • S S L S S L S																				
1262:	Y	C	V	E	D	T	P	I	C	F	S	R	C	S	S	L	S	S	L	S
1378:	H	Y	V	Q	E	T	P	L	M	F	S	R	C	T	S	V	S	S	L	D
1492:	F	A	T	E	S	T	P	D	G	F	S	C	S	S	S	L	S	A	L	S
1643:	Y	C	V	E	G	T	P	I	N	F	S	T	A	T	S	L	S	D	L	T
1848:	T	P	I	E	G	T	P	Y	C	F	S	R	N	D	S	L	S	S	L	D
1953:	F	A	I	E	N	T	P	V	C	P	S	H	N	S	S	L	S	S	L	S
2013:	F	H	V	E	D	T	P	V	C	F	S	R	N	S	S	L	S	S	L	S

Numbers denote the first amino acid of each repeat. The consensus sequence at the top reflects a majority amino acid at a given position.

International Application No: **REC'D 16 MAR 1992****MICROORGANISMS****WIPO PCT**Optional Sheet in connection with the microorganism referred to on page 22, line 23 of the description \***A. IDENTIFICATION OF DEPOSIT \***Further deposits are identified on an additional sheet ☐ \*

Name of depositary institution \*

**NATIONAL COLLECTION OF INDUSTRIAL AND MARINE BACTERIA (NCIMB)**

Address of depositary institution (including postal code and country) \*

**23 St. Machar Drive  
Aberdeen AB2 1RY, Scotland  
United Kingdom**

Date of deposit \*

**17 December 1990**

Accession Number \*

**NCIMB 40353****B. ADDITIONAL INDICATIONS \*** (leave blank if not applicable). This information is continued on a separate attached sheet ☐**Saccharomyces cerevisiae SC/37H64**

In respect to those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only the issue of such a sample to an expert nominated by the person requesting the sample. (Rule 28(4) EPC)

**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE \*** (If the indications are not for all designated States)**D. SEPARATE FURNISHING OF INDICATIONS \*** (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later \* (Specify the general nature of the indications e.g. - Accession Number of Deposit \*)

**E.** ☒ This sheet was received with the international application when filed (to be checked by the Receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is

was

(Authorized Officer)

11 Rec'd PCT/PTC 02 APR 1990

International Application No: PCT/

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# MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 22 and 23 of the description.

## A. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet ☒.

Name of depositary institution:

NATIONAL COLLECTION OF INDUSTRIAL AND MARINE BACTERIA (NCIMB)

Address of depositary institution (including postal code and country):

23 St. Machar Drive  
Aberdeen AB2 1RY, Scotland  
United Kingdom

Date of deposit:

17 December 1990

Accession Number:

NCIMB 40353

B. ADDITIONAL INDICATIONS (leave blank if not applicable). This information is continued on a separate attached sheet ☒.

*Saccharomyces cerevisiae* SC/37HG4  
For the designation of Denmark, the applicant request that the sample of the microorganism only be furnished to an expert until the application has been accepted or finally decided without having been accepted (Section 22(7)).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (Specify the general nature of the indications and Accession Number of Deposit)

☒ This sheet was received with the international application when filed (to be checked by the receiving Office)

*Shuley Harris*  
(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is

was

(Authorized Officer)



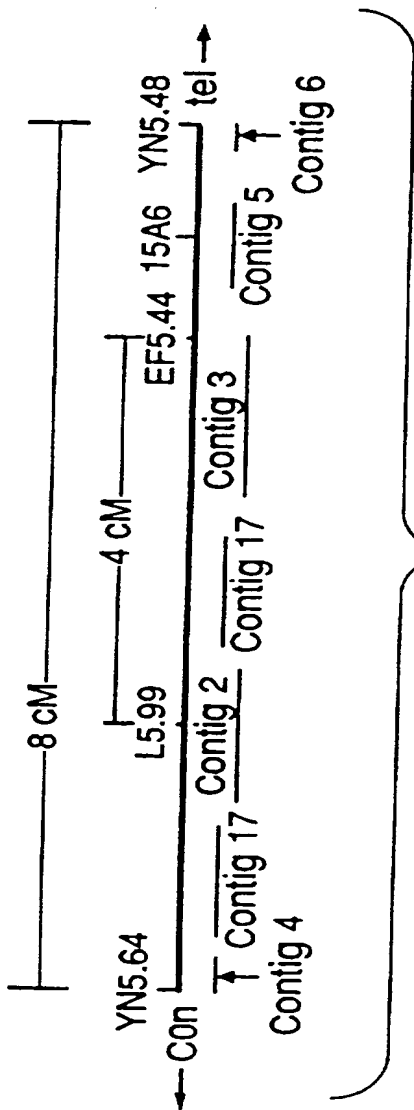


FIG. 1A

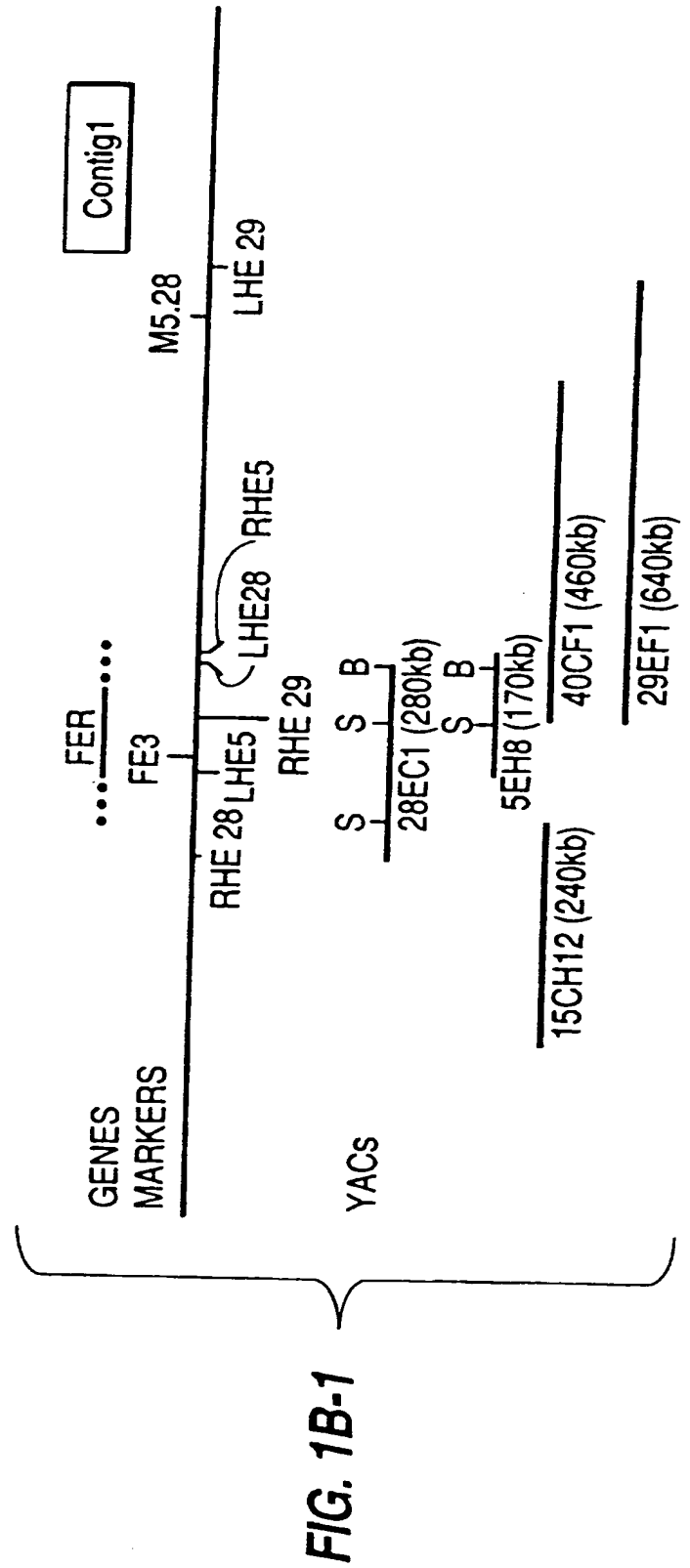
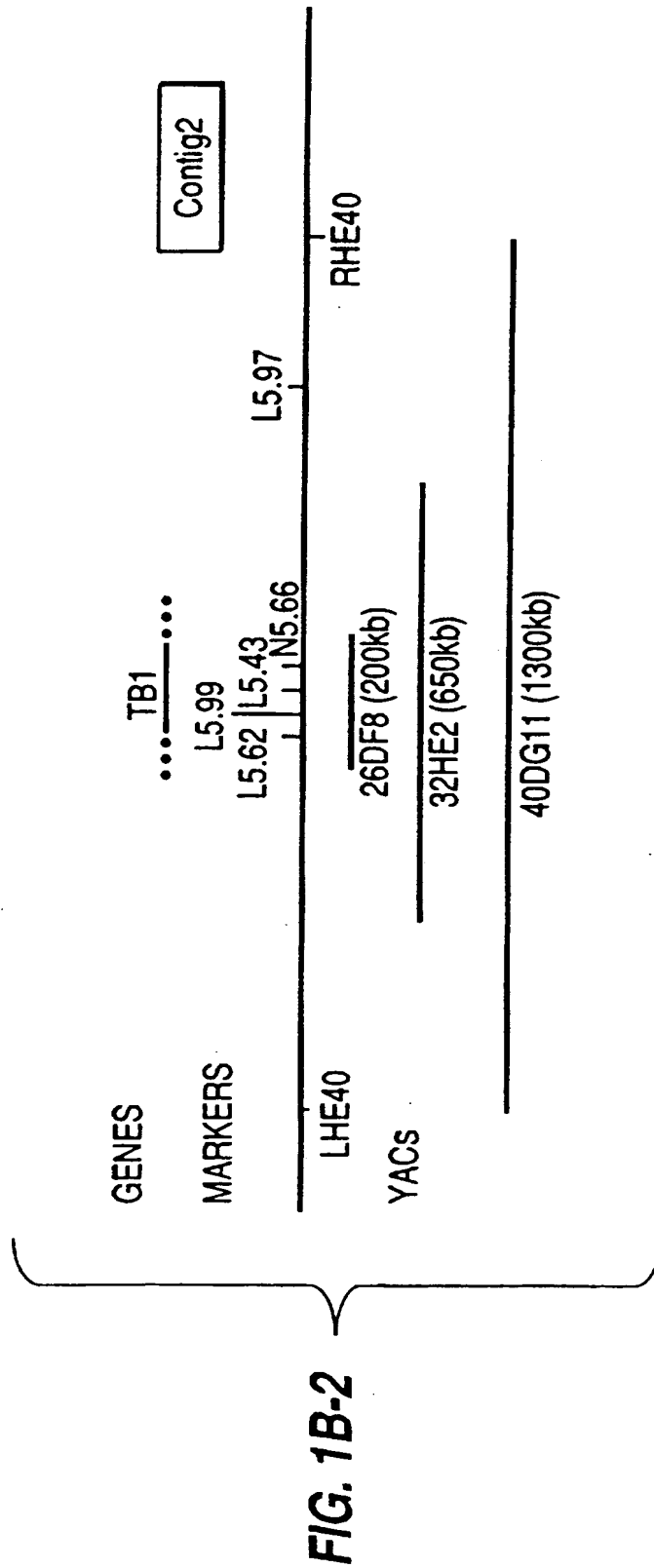
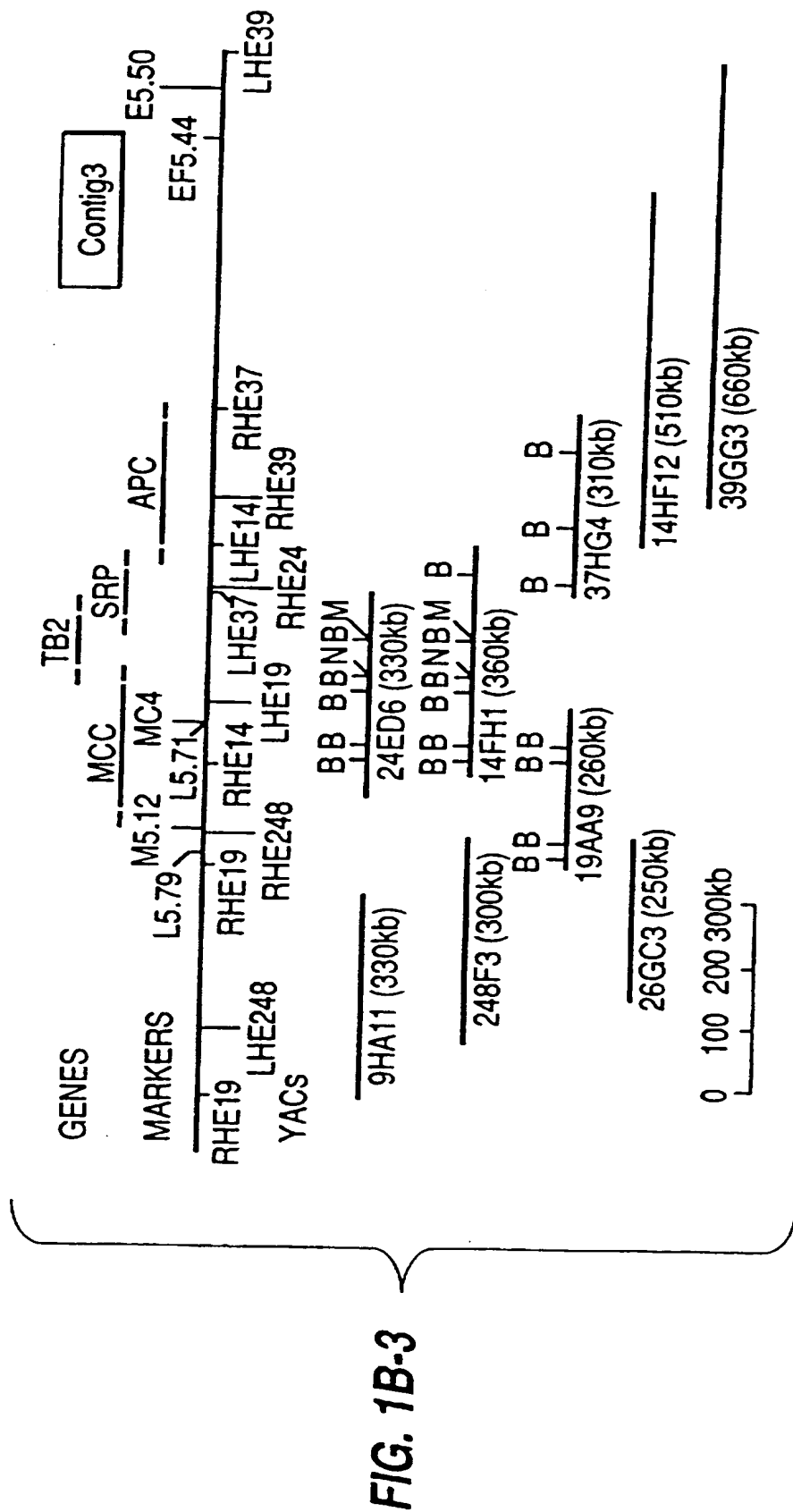


FIG. 1B-1

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**FIG. 2A****TB1 AMINO ACID SEQUENCE**

VAPVVVSGR APRHPAPAAM HPRRPOGFDG LGYRGGAROE QGFGGAFPAR SFSTGSDLGH 60  
WVTTPPDIPG SRNLHWGEKS PPYGVPTTST PYEGPTEEPF SSGGGGSVQG QSSEQLNRFA 120  
GFGIGLASLF TENVLAHPCI VLRROCQVNY HAQHYHLTPF TVINIMYSFN KTOGPRALWK 180  
GMGSTFIVQG VTLGAEGIIS EFTPLPREVL HKWSPKQIGE HLLKSLTYV VAMPFYSASL 240  
IETVQSEIIR DNTGILECVK EGIGRVIGMG VPHSKRLLPL LSLIFPTVLH GVLHYIISV 300  
IQKFVLLILK RKTYNHSLAE STSPVQSMLO AYFPELIANF AASLCSDVIL YPLETVLHRL 360  
HIQGTIRTIID NTDLGYEVLP INTQYEGMRD CINTIRQEEG VFGFYKGFGA VIIQYTLHAA 420  
VLQITKIISY TLLO 434

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**FIG. 2B****TB2 AMINO ACID SEQUENCE**

ELRRFRDRLH EKNCHTDLLA KLEAKTGVNR SFIALGVIGL VALYLVFGYG ASLLCNLIGF 60  
GYPAYISIKA IESPNKEDDT QWLTYNVVYG VFSIAEFFSD IFLSNFPFYY ILKCGFLLWC 120  
MAPSPSGAE LLYKRIIRPF FLKHESQMSD VVKDLKDKAK ETADAITKEA KKATVNLLGE 180  
EKKST 185

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## FIG. 3A

MAAASYDQLL	KQVEALKMEN	SNLRQLEDN	SNHLTKLETE	ASNKMEVLKQ	LOGSIEDEAM	60
ASSGQIDLE	RLKELNLDSS	NFPGVKLRSK	MSLSYGSRE	GSVSSRSSEC	SPVPMGSFPR	120
RGFVNGSRES	TGYLEELEKE	RSLLADLDK	EEKEKDWYA	QLONLTKRID	SLLTENFSLQ	180
TDMTRRQLEY	EARQIRVAME	EOLGTCODME	KRAORRIARI	QOIEKDILRI	RQLLOSQATE	240
AERSSQNKHE	TGSHDAERQW	EGQGVGEINM	ATSGNGQGST	TRMDHETASV	LSSSSTHSAP	300
RRLTSHLGTK	VEMVYSLLSM	LGTHDKDDMS	RTLAMSSSO	DSCISMROSG	CLPLLIQLLH	360
GNDKDSVLLG	NSRGSKEARA	RASAALHNII	HSQPDOKRGR	REIRVLHLE	QIRAYCETCW	420
EWQEAHEPGM	DODKNPMPAP	VEHQICPAVC	VLKLSFDEE	HRHAMNELGG	LOAIAELLQV	480
DCEHYGLTND	HYSITLRRYA	GHALTNLTFG	DVANKATLCS	MKGCHRALVA	QLKSESEDLO	540
QVIASVLRNL	SWRADVNSKK	TLREVGSKA	LMECALEVKK	ESTLKSIVLSA	LWNLSAHCTE	600
NKADICAVDG	ALAFVLGTLT	YRSQNTLAI	IESGGGILRN	VSSLIATNED	HRQILRENNC	660
LQTLLOHLKS	HSLTIVSNAC	GTLWNLSARN	PKDQEAALWDM	GAVSHLKNLI	HSKHKMIAMG	720
SAAALRNLM	NRPAKYKDAN	IMSPGSSLPS	LHVRKOKALE	AELDAQHLE	TFDNIDNLS	780
KASHRSKQRH	KQSLYGDYVF	DTNRHDDNRS	DNFNTGNMTV	LSPYLNTTVL	PSSSSSRGSL	840
DSSRSEKDRS	LERERGIGLG	NYHPATENPG	TSSKRGLOIS	TTAAQIAKVM	EEVSAIHTSQ	900
EDRSSGSTTE	LHCVTDERNA	LRRSSAAATH	SNTYNFTKSE	NSNRITCSMPY	AKLEYKRSSN	960
DSLNSVSSSD	GYGKRGOMKP	SIESYSEDDE	SKFCSYGQYP	ADLAHKIHS	NHMDNDGEL	1020
DTPINYSKY	SDEQLNSGRQ	SPSONERWAR	PKHIIIEDEIK	OSEORQRNQ	STTYPVYTES	1080
TDDKHLKFQ	HFGQCECVSP	YRSRGANGSE	TNRVGSNHGI	NONVSQSLCQ	EDDYEDDKPT	1140
NYSERYSEEE	QHEEEERPTN	YSIKYNEEKR	HVDQPIDYSL	KYATDIPSSQ	KQSFSFSKSS	1200
SGOSSKTEHM	SSSSENTSTP	SSNAKRONQL	HPSSAQRSG	QPOKAATCKV	SSINQETIQ	1260
YCVEDTPICF	SRCSSLSLS	SAEDEIGCNQ	TTODPOSANT	LQIAEIKEKI	GTRSAEDPVS	1320
EVPAVSQHPR	TKSSRLQSS	LSSESARHKA	VEFSSGAKSP	SKSGAQTPKS	PPEHYVOETP	1380
LMFSRCTSVS	SLDSFESRSI	ASSVQSEPCS	GMVSGIISPS	DLPOSPGQTM	PPSRSKTPPP	1440

FIG. 3B

LMFSRCTSVS	SLDSFESRSI	ASSVQSEPCS	GHVSGIISPS	DLDPSPGQTM	PPSRSKTPPP	1440
PPQTAQTKRE	VPKNKAPTAE	KRESGPKQAA	VNAAVQVRVQV	LPDADTLHF	ATESTPOGFS	1500
CSSSLSALS	DEPFIQKDV	LRIMPPVQEN	DNGNETESEQ	PKESNENQEK	EAEKTIIDSEK	1560
DLDDSDDDD	IEILEECIIS	AMPTKSSRKA	KKPAQTASKL	PPPVARKPSQ	LPVYKLLPSQ	1620
NRLQPKHVS	FTPGDDMPRV	YCVGTPINF	STATSLDOLT	IESPPNELAA	GEGVRGGAQS	1680
GEFEKRDITP	TEGRSTDEAQ	GGKTSSVTIP	ELDONKAEEG	DILAECINSA	MPKGKSHKPF	1740
RVKKIMDOVQ	QASASSAPN	KNOLGKXXX	PTSPVKPIPO	NTEYRTRVRK	NADSKNHLNA	1800
ERVFSDNKDS	KKQNLKNNSK	DFNDKLPNNE	DRVRSFAFD	SPHHYTPIEG	TPYCFSRNDS	1860
LSSLOFDDDD	VDLSREKAEL	RKAKENKESE	AKVTSHTELT	SNOOSANKTO	AIAKQPINRG	1920
OPKPILOKOS	TFPOSSKDIP	DRGAATDEKL	QNFAIENTPV	CFSHNSLSS	LSDIDQENNM	1980
KENEPKIKETE	PPDSQGEPSK	POASGYAPKS	FHVEDTPVCF	SRNSSLSLS	IDSEDDLLOE	2040
CISSAMPKKK	KPSRLKGDNE	KHSPRNMGGI	LGEDLTDLK	DIQRPOSEHG	LSPOSENFOW	2100
KAIQEGANSI	VSSLHQAAA	ACLSRQASSD	SDSILSLKSG	ISLGSPPFHLT	PDQEEKPFTS	2160
NKGPRILKPG	EKSTLETKKI	ESESKGKGG	KKVYKSLITG	KVRSNSEISG	OMKQPLOANM	2220
PSISRGRTHI	HIPGVRNSSS	STSPVSKKGP	PLKTPASKSP	SEGQTATTSP	RGAKPSVKSE	2280
LSPVARQTSQ	IGGSSKAPSR	SGSRDSTPSR	PAQQPLSRPI	QSPGRNSISP	GRNGISPPNK	2340
LSQLPRTSSP	STASTKSSGS	GKMSYTSRGR	OMSOQNLTKQ	TGLSKNASSI	PRSESASKGL	2400
NQMNNGNGAN	KKVELSRMSS	TKSSGSESDR	SERPVLVROS	TFIKEAPSPT	LRRKLEESAS	2460
FESLSPSSRP	ASPTRSOAQT	PVLSPSLPDM	SLSTHSSVOA	GGWRKLPNNL	SPTIEYNDGR	2520
PAKRHDIIARS	HSESPSRLPI	NRSGETKREH	SKHSSSLPRV	STWRRTGSSS	SILSASSESS	2580
EKAKSEDEKH	VNSISGTKQS	KENQVSAGKT	WRKIKENEFS	PTNSTSQTVS	SGATNGAESK	2640
TLIYQMAPAV	SKTEDVWVRI	EDCPINNPRS	GRSPTGNTPP	VIDSVSEKAN	PNIKDSKDNO	2700
AKQNVGNGSV	PMRTVGLENR	LNSFIOVDAP	DOKGTEIKPG	QNNPVPVSET	NESSIVERTP	2760
FSSSSSSKHS	SPSGTVAARV	TPFNYNPSPR	KSSADSTARS	PSQIPTPVNN	NTKKRDSKTD	2820
STESSGTOSP	KRHSGSYLVT	SV				2842

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**FIG. 4A**

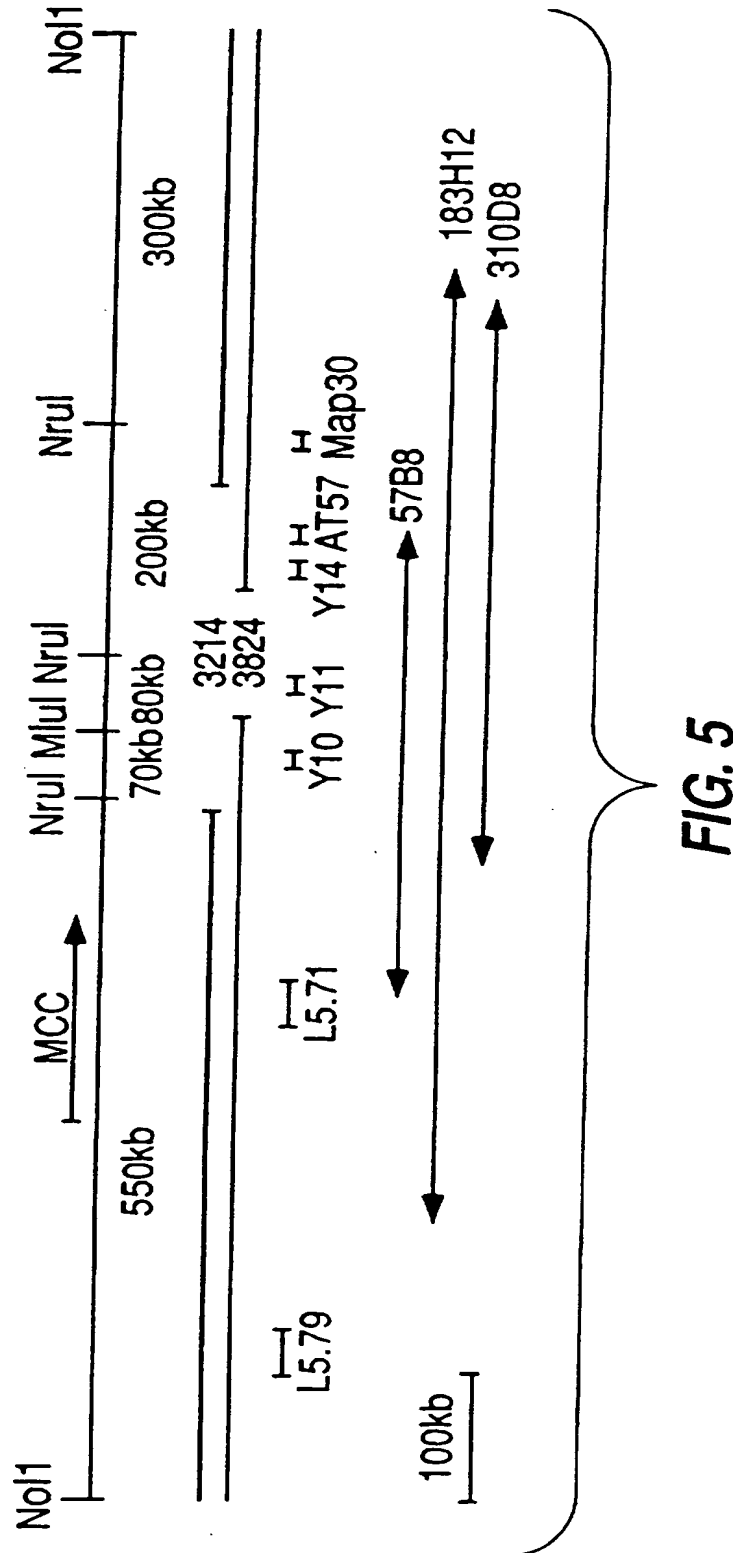
APC	203	LGTCDDMEKRAQRRIARIQQIEKDILRIQL	233
		::	
RAL2	576	LTGAKGLQLRALRRRIARIEGGTAISPTSPL	606

**FIG. 4B**

APC	453	MKLSFDEEHRHAMNELGGLOAIAELLQVD	481
		:               :   :       :       :	
M3 MACHR	249	LYWRIYKETEKRTKELAGLOASGTEAETE	277
		:               :	
MCC	220	LYPNLAEEERSRWEKELAGREENESLTAM	248
		:         : :         :	
APC	453	MKLSFDEEHRHAMNELGGLOAIAELLQVD	481



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**FIG. 6A**

GCA GTC GCC GCT CCA GTC TAT CCG GCA	CTA GGA ACA GCC CCG GGN GGC GAG	55
Ala Val Ala Ala Pro Val Tyr Pro Ala	Leu Gly Thr Ala Pro Gly Gly Glu Thr	109
GTC CCC GCC ATG TCT GCG GCC ATG AGG	GAG AGG TTC GAC CCG TTC CTG CAC GAG	163
Val Pro Ala MET Ser Ala Ala MET Arg	Glu Arg Phe Asp Arg Phe Leu His Glu	217
AAG AAC TGC ATG ACT GAC CTT CTG GCC	AAG CTC GAG GCC AAA ACC GGC GTG AAC	271
Lys Asn Cys MET Thr Asp Leu Leu Ala	Lys Leu Glu Ala Lys Thr Gly Val Asn	325
AGG AGC TTC ATC GCT CTT GGT GTC ATC	GGA CTG GTG GCC TTG TAC CTG GTG TTC	379
Arg Ser Phe Ile Ala Leu Gly Val Ile	Gly Leu Val Ala Leu Tyr Leu Val Phe	433
GGT TAT GGA GCC TCT CTC CTC CTC TGC	AAC GGA TTT GGC TAC CCA GCC TAC	487
Gly Tyr Gly Ala Ser Leu Leu Cys Asn	Leu Ile Gly Phe Gly Tyr Pro Ala Tyr	541
ATC TCA ATT AAA GCT ATA GAG AGT CCC	AAC AAA GAA GAT ACC CAG TGG CTG	
Ile Ser Ile Lys Ala Ile Glu Ser Pro	Asn Lys Glu Asp Thr Gln Trp Leu	
ACC TAC TGG GTA GTG TAT GGT GTG TTT	AGC ATT GCT GAA TTC TTC TCT GAT ATC	
Thr Tyr Trp Val Val Tyr Gly Val Phe	Ser Ile Ala Glu Phe Phe Ser Asp Ile	
TTC CTG TCA TGG TTC CCC TTC Phe Tyr	MET Leu Lys Cys Gly Phe Leu Trp	
Phe Leu Ser Trp Phe Pro Phe Tyr	460	
TGC ATG GCC CCG AGC CCT TCT AAT GGG	GCT GAA CTG CTC TAC AAG CGC ATC ATC	
Cys MET Ala Pro Ser Pro Ser Asn Gly	Ala Glu Leu Leu Tyr Lys Arg Ile Ile	
CGT CCT TTC TTC CTG AAG CAC GAG TCC	CAG ATG GAC AGT GTG GTC AAG GAC CTT	
Arg Pro Phe Phe Leu Lys His Glu Ser	Gln MET Asp Ser Val Val Lys Asp Leu	

## FIG. 6B

AAA GAC AAG TCC AAA GAG ACT GCA GAT GCC ATC ACT AAA GAA GCG AAG AAA GCT	568	595
Lys Asp Lys Ser Lys Glu Thr Ala Asp Ala Ile Thr Lys Glu Ala Lys Lys Ala		
ACC GTG AAT TTA CTG GGT GAA GAA AAG AAG AGC ACC TAA ACC AGA	622	
Thr Val Asn Leu Leu Gly Glu Glu Lys Lys Ser Thr		
CTAAACCAGA CTGGATGGAA ACTTCCTGCC CTCTCTGTAC CTTCCTACTG GAGCTTGATG TTATATTAGG	640 650 660 670 680 690 700	
710 720 730 740 750 760 770		
GACTGTGGTA TAATTATTTT AATAATGTTG CCTTGGAAAC ATTTTGTAGA TATTAAAGAT TCGAATGTGT		
780 790 800 810 820 830 840		
TGTAAGTTTC TTTGCTTACT TTTACTGTCT ATATATATAG GGAGCACTTT AAACCTTAATG CAGTGGGCAG		11/48
850 860 870 880 890 900 910		
TGTCCACGTT TTTGGAAAAT GTATTTTGCC TCTGGGTAGG AAAAGATGTA TGTTGCTATC CTGCAGGAAA		
920 930 940 950 960 970 980		
TATAAACTTA AAATAAAATT ATATACCCCA CAGGCTGTGT ACTTTACTGG GCTCTCCCTG CACGSATTTT		
990 1000 1010 1020 1030 1040 1050		
CTCTGTAGTT ACATTTAGGR TAATCTTTAT GGTTCTACTT CCTRTAAATGT ACAATTTTAT ATAATTCNGR		
1060 1070 1080 1090 1100 1110 1120		
AATGTTTTTA ATGTATTTGT GCACATGTAC ATATGGAAAT GTTACTGTCT GACTACANCA TGCATCATGC		
1130 1140 1150 1160 1170 1180 1190		
TCATGGGGAG GGAGCAGGGG AAGGTTGTAT GTGTCATTTA TAACTTCTGT ACAGTAAGAC CACCTGCCAA		
1200 1210 1220 1230 1240 1250 1260		
AAGCTGGAGG AACCATTGTG CTGGTGTGGT CTACTAAATA ATACTTTAGG AAATACGTGA TTAATATGCA		
1270 1280 1290 1300 1310 1320 1330		
AGTGAACAAA GTGAGAAATG AAATCGAATG GAGATTGGCC TGGTTGTTTC CGTAGTATAT GGCATATGAA		
1340 1350 1360 1370 1380 1390 1400		

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## FIG. 6C

TACCAGGATA	GCTTTATAAA	GCAGTTAGTT	AGTTAGTTAC	TCACTCTAGT	GATAAATCGG	GAAATTTACA
1410	1420	1430	1440	1450	1460	1470
CACACACACA	CACACACACA	CACACACACA	CACACACACA	CACACACACA	GAGTACCCTG	TAACTCTCAA
1480	1490	1500	1510	1520	1530	1540
TTCCCTGAAA	AACTAGTAAT	ACTGTCTTAT	CTGCTATAAA	CTTTACATAT	TTGTCTATTG	TCAAGATGCT
1550	1560	1570	1580	1590	1600	1610
ACANTGGAMN	CCATTCTGG	TTTTATCTTC	ANAGSGGAGA	NACATGTTGA	TTTAGTCTTC	TTTCCCCAATC
1620	1630	1640	1650	1660	1670	1680
TTCTTTTTTA	AMCCAGTTN	AGGMNCTTCT	GRAGATTGY	CCACCCTCTGA	TTACATGTAT	GTTCTYGTTC
1690	1700	1710	1720	1730	1740	1750
GTATCATKAG	CAACAACATG	CTAATGRCGA	CACCTAGCTC	TRAGMGCAAT	TCTGGGAGAN	TGARAGGNWG
1760	1770	1780	1790	1800	1810	1820
TATARAGTMN	CCCATAATCT	GCTTGGCAAT	AGTTAAGTCA	ATCTATCTTC	AGTTTTTCTC	TGGCCTTTAA
1830	1840	1850	1860	1870	1880	1890
GGTCAAACAC	AAGAGGCTTC	CCTAGTTTAC	AAGTCAGAGT	CACTTGTAGT	CCATTTAAAT	GCCCTCATCC
1900	1910	1920	1930	1940	1950	1960
GTATTCTTTG	TGTTGATAAG	CTGCACAKGA	CTACATAGTA	AGTACAGANC	AGTAAAGTTA	ANNCGGATGT
1970	1980	1990	2000	2010	2020	2030
CTCCATTGAT	CTGCCAANTC	GNTATAGAGA	GCAATTGTGC	TGGACTAGAA	AATCTGAGTT	TTACACCATA
2040	2050	2060	2070	2080	2090	2100
CTGTTAAGAG	TCCTTTTGAA	TTAAACTAGA	CTAAACAACG	TGTATAACTA	AACTAACAAG	ATTAAATATC
2110	2120	2130	2140	2150	2160	2170
CAGCCAGTAC	AGTATTTTTT	AAGGCAATAA	AAGATGATTA	GCTCACCTTG	AGNTAACAAAT	CAGGTAAGAT
2180	2190	2200	2210	2220	2230	2240
CATNACAATG	TCTCATGATG	TNAANAATAT	TAAAGATATC	AATACTAAGT	GACAGTATCA	CNNCTAATAT

## FIG. 6D

2250	2260	2270	2280	2290	2300	2310
AATATGGATC	AGAGCATTTA	TTTTGGGGAG	GAAAAACAGTG	GTGATTACCG	GCATTTTATT	AAACTTAAAA
2320	2330	2340	2350	2360	2370	2380
CTTTGTAGAA	AGCAAAACAAA	ATTGTTCTTG	GGAGAAAATC	AACTTTTAGA	TTAAAAAAAT	TTTAAAGTAWC
2390	2400	2410	2420	2430	2440	2450
TAGGAGTATT	TAAATCCTTT	TCCCATAAAT	AAAAGTACAG	TTTTTCTTGGT	GGCAGAAATGA	AAATCAGCAA
2460	2470	2480	2490	2500	2510	2520
CNTCTAGCAT	ATAGACTATA	TAATCAGATT	GACAGCATAT	AGAAATATATT	ATCAGACAAG	ATGAGGAGGT
2530	2540	2550	2560	2570	2580	2590
ACAAAAGTTA	CTATTGCTCA	TAATGACTTA	CAGGCTAAAA	NTAGNTNTAA	AATACTATAT	TAAATTCTGA
2600	2610	2620	2630	2640	2650	2660
ATGCAATTTT	TTTTTGTTC	CTTGAGACCA	AAATTTAAGT	TAACTGTTGC	TGGCAGTCTA	AGTGTAATG <sup>3/48</sup>
2670	2680	2690	2700	2710	2720	2730
TTAACAGCAG	GAGAAGTTAA	GAATTGAGCA	GTTCTGTTGC	ATGATTTCCTC	AAATGAAATA	CTGCCCTTGGC
2740	2750	2760	2770	2780	2790	2800
TAGAGTTTGA	AAAACATAAT	GAGCCTGTGC	CTGGCTAGAA	AACAAGCGTT	TATTTGAATG	TGAATAGTGT
2810	2820	2830	2840	2850	2860	2870
TTCAAAGGTA	TGTAGTTACA	GAATTCCTAC	CAAAACAGCTT	AAATTCTTCA	AGAAAGAATT	CCTGCAGCAG
2880	2890	2900	2910	2920	2930	2940
TTATTCCCTT	ACCTGAAGGC	TTCAATCAAT	TGGATCAACA	ACTGCTACTC	TCGGGAAGAC	TCCTCTACTC
2950	2960	2970	2980	2990	3000	3010
ACAGCTGAAG	AAAATGAGCA	CACCCCTTAC	ACTGTTATCA	CCTATCCTGA	AGATGTGATA	CACTGAATGG
3020	3030	3040	3050	3060	3070	3080
AAATAAATAG	ATGTAATAA	AATTGAGWTC	TCATTTAAAA	AAAACCATGT	GCCCAATGGG	AAAATGACCT
3090	3100	3110	3120	3130	3140	3150
CATGTTGTGG	TTTAAACAGC	AACTGCACCC	ACTAGCACAG	CCCATTGAGC	TANCCATAT	ATACATCTCT
3160						
GTCAGTGCCC	CTC					

## FIG. 7A

27 54  
 GGA CTC GGA AAT GAG GTC CAA GGG TAG CCA AGG ATG GCT GCA GCT TCA TAT GAT  
 Gly Leu Gly Asn Glu Val Gln Gly . Pro Arg MET Ala Ala Ser Tyr Asp

81 108  
 CAG TTG TTA AAG CAA GTT GAG GCA CTG AAG ATG GAG AAC TCA AAT CTT CGA CAA  
 Gln Leu Leu Lys Gln Val Glu Ala Leu Lys MET Glu Asn Ser Asn Leu Arg Gln

135 162  
 GAG CTA GAA GAT AAT TCC AAT CAT CTT ACA AAA CTG GAA ACT GAG GCA TCT AAT  
 Glu Leu Glu Asp Asn Ser Asn His Leu Thr Lys Leu Glu Thr Glu Ala Ser Asn

189 216  
 ATG AAG GAA GTA CTT AAA CAA CTA CAA GGA AGT ATT GAA GAT GAA GCT ATG GCT  
 MET Lys Glu Val Leu Lys Gln Leu Gln Gly Ser Ile Glu Asp Glu Ala MET Ala

243 270  
 TCT TCT GGA CAG ATT GAT TTA TTA GAG CGT CTT AAA GAG CTT AAC TTA GAT AGC  
 Ser Ser Gly Gln Ile Asp Leu Leu Glu Arg Leu Lys Glu Leu Asn Leu Asp Ser

297 324  
 AGT AAT TTC CCT GGA GTA AAA CTG CGG TCA AAA ATG TCC CTC CGT TCT TAT GGA  
 Ser Asn Phe Pro Gly Val Lys Leu Arg Ser Lys MET Ser Leu Arg Ser Tyr Gly

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## FIG. 7B

AGT AAT TTC CCT GGA GTA AAA CTG CGG TCA AAA ATG TCC CTC CGT TCT TAT	297	324
Ser Asn Phe Pro Gly Val Lys Leu Arg Ser Lys MET Ser Leu Arg Ser Tyr Gly		GGA
AGC CGG GAA GGA TCT GTA TCA AGC CGT TCT GGA GAG TGC AGT CCT GTT CCT	351	378
Ser Arg Glu Gly Ser Val Ser Ser Arg Ser Gly Glu Cys Ser Pro Val Pro MET		ATG
GGT TCA TTT CCA AGA AGA GGG TTT GTA AAT GGA AGC AGA GAA AGT ACT GGA	405	432
Gly Ser Phe Pro Arg Arg Gly Phe Val Asn Gly Ser Arg Glu Ser Thr Gly Tyr		TAT
TTA GAA GAA CTT GAG AAA GAG AGG TCA TTG CTT CTT GCT GAT CTT GAC AAA	459	486
Leu Glu Glu Leu Leu Lys Glu Arg Ser Leu Leu Leu Ala Asp Leu Asp Lys Glu		GAA
GAA AAG GAA AAA GAC TGG TAT TAC GCT CAA CTT CAG AAT CTC ACT AAA AGA	513	540
Glu Lys Glu Lys Asp Trp Tyr Tyr Ala Gln Leu Leu Gln Asn Leu Thr Lys Arg Ile		ATA
	567	594

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## FIG. 7C

GAT AGT CTT CCT TTA ACT GAA AAT TTT TCC TTA CAA ACA GAT TTG ACC AGA AGG  
 Asp Ser Leu Pro Leu Thr Glu Asn Phe Ser Leu Gln Thr Asp Leu Thr Arg Arg

621  
 CAA TTG GAA TAT GAA GCA AGG CAA ATC AGA GTT GCG ATG GAA GAA CAA CTA GGT  
 Gln Leu Glu Tyr Glu Ala Arg Gln Ile Arg Val Ala MET Glu Gln Leu Gly 648

675  
 ACC TGC CAG GAT ATG GAA AAA CGA GCA CAG CGA AGA ATA GCC AGA ATT CAG CAA  
 Thr Cys Gln Asp MET Glu Lys Arg Ala Gln Arg Arg Ile Ala Arg Ile Gln Gln 702

729  
 ATC GAA AAG GAC ATA CTT CGT ATA CGA CAG CTT TTA CAG TCC CAA GCA ACA GAA  
 Ile Glu Lys Asp Ile Leu Arg Ile Arg Gln Leu Gln Ser Gln Ala Thr Glu 756

783  
 GCA GAG AGG TCA TCT CAG AAC AAG CAT GAA ACC GGC TCA CAT GAT GCT GAG CGG  
 Ala Glu Arg Ser Ser Gln Asn Lys His Glu Thr Gly Ser His Asp Ala Glu Arg 810

837  
 CAG AAT GAA GGT CAA GGA GTG GGA GAA ATC AAC ATG GCA ACT TCT GGT AAT GGT  
 Gln Asn Glu Gly Gln Gly Val Gly Glu Ile Asn MET Ala Thr Ser Gly Asn Gly 864

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**FIG. 7D**

CAG GGT TCA ACT ACA CGA ATG GAC CAT GAA ACA GCC AGT GTT TTG AGT TCT AGT 918  
 Gln Gly Ser Thr Thr Arg MET Asp His Glu Thr Ala Ser Val Leu Ser Ser Ser  
 891

AGC ACA CAC TCT GCA CCT CGA AGG CTG ACA AGT CAT CTG GGA ACC AAG GTG GAA 972  
 Ser Thr His Ser Ala Pro Arg Arg Leu Thr Ser His Leu Gly Thr Lys Val Glu

945

ATG GTG TAT TCA TTG TTG TCA ATG CTG GGT ACT CAT GAT AAG GAT GAT ATG TCG 1026  
 MET Val Tyr Ser Leu Leu Ser MET Leu Gly Thr His Asp Lys Asp MET Ser

999

CGA ACT TTG CTA GCT ATG TCT AGC TCC CAA GAC AGC TGT ATA TCC ATG CGA CAG 1080  
 Arg Thr Leu Leu Ala MET Ser Ser Ser Gln Asp Ser Cys Ile Ser MET Arg Gln

1053

TCT GGA TGT CTT CCT CTC CTC ATC CAG CTT TTA CAT GGC AAT GAC AAA GAC TCT 1134  
 Ser Gly Cys Leu Pro Leu Leu Ile Gln Leu Leu His Gly Asn Asp Lys Asp Ser

1107

GTA TTG TTG GGA AAT TCC CGG GGC AGT AAA GAG GCT CGG GCC AGG GCC AGT GCA 1188

1161

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## FIG. 7E

Val Leu Leu Gly Asn Ser Arg Gly Ser Lys Glu Ala Arg Ala Ser Ala

1215  
GCA CTC CAC AAC ATC ATT CAC TCA CAG CCT GAT GAC AAG AGA GGC AGG CGT GAA 1242  
Ala Leu His Asn Ile Ile His Ser Gln Pro Asp Lys Arg Gly Arg Arg Glu

1269  
ATC CGA GTC CTT CAT CTT TTG GAA CAG ATA CGC GCT TAC TGT GAA ACC TGT TGG 1296  
Ile Arg Val Leu His Leu Leu Glu Glu Gln Ile Arg Ala Tyr Cys Glu Thr Cys Trp

1323  
GAG TGG CAG GAA GCT CAT GAA CCA GGC ATG GAC CAG GAC AAA AAT CCA ATG CCA 1350  
Glu Trp Gln Glu Ala His Glu Pro Gly MET Asp Gln Asp Lys Asn Pro MET Pro

1377  
GCT CCT GTT GAA CAT CAG ATC TGT CCT GCT GTG TGT GTT CTA ATG AAA CTT TCA 1404  
Ala Pro Val Glu His Gln Ile Cys Pro Ala Val Cys Val Leu MET Lys Leu Ser

1431  
TTT GAT GAA GAG CAT AGA CAT GCA ATG AAT GAA CTA GGG GGA CTA CAG GCC ATT 1458  
Phe Asp Glu Glu His Arg His Ala MET Asn Glu Leu Gly Gly Leu Gln Ala Ile

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**FIG. 7F**

1485  
 GCA GAA TTA TTG CAA GTG GAC TGT GAA ATG TAT GGG CTT ACT AAT GAC CAC TAC  
 Ala Glu Leu Leu Gln Val Asp Cys Glu MET Tyr Gly Leu Thr Asn Asp His Tyr 1512

1539  
 AGT ATT ACA CTA AGA CGA TAT GCT GGA ATG GCT TTG ACA AAC TTG ACT TTT GGA  
 Ser Ile Thr Leu Arg Arg Tyr Ala Gly MET Ala Leu Thr Asn Leu Thr Phe Gly 1566

1593  
 GAT GTA GCC AAC AAG GCT ACG CTA TGC TCT ATG AAA GGC TGC ATG AGA GCA CTT  
 Asp Val Ala Asn Lys Ala Thr Leu Cys Ser MET Lys Gly Cys MET Arg Ala Leu 1620

1647  
 GTG GCC CAA CTA AAA TCT GAA AGT GAA GAC TTA CAG CAG GTT ATT GCA AGT GTT  
 Val Ala Gln Leu Lys Ser Glu Ser Glu Asp Leu Gln Val Ile Ala Ser Val 1674

1701  
 TTG AGG AAT TTG TCT TCG CGA GAT GTA AAT AGT AAA AAG ACG TTG CGA GAA  
 Leu Arg Asn Leu Ser Trp Arg Ala Asp Val Asn Ser Lys Lys Thr Leu Arg Glu 1728

1755  
 GTT GGA AGT GTG AAA GCA TTG ATG GAA TGT GCT TTA GAA GTT AAA AAG GAA TCA  
 Val Gly Ser Val Lys Ala Leu MET Glu Cys Ala Leu Glu Val Lys Lys Glu Ser 1782

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## FIG. 7G

1809  
ACC CTC AAA AGC GTA TTG AGT GCC TTA TGG AAT TTG TCA GCA CAT TGC ACT GAG  
Thr Leu Lys Ser Val Leu Ser Ala Leu Trp Asn Leu Ser Ala His Cys Thr Glu 1836

1863  
AAT AAA GCT GAT ATA TGT GCT GTA GAT GGT GCA CTT GCA TTT TTG GTT GGC ACT  
Asn Lys Ala Asp Ile Cys Ala Val Asp Gly Ala Leu Ala Phe Leu Val Gly Thr 1890

1917  
CTT ACT TAC CGG AGC CAG ACA AAC ACT TTA GCC ATT ATT GAA AGT GGA GGT GGG  
Leu Thr Tyr Arg Ser Gln Thr Asn Thr Leu Ala Ile Ile Glu Ser Gly Gly Gly 1944

1971  
ATA TTA CGG AAT GTG TCC AGC TTA ATA GCT ACA AAT GAG GAC CAC AGG CAA ATC  
Ile Leu Arg Asn Val Ser Ser Leu Ile Ala Thr Asn Glu Asp His Arg Gln Ile 1998

2025  
CTA AGA GAG AAC AAC TGT CTA CAA ACT TTA TTA CAA CAC TTA AAA TCT CAT AGT  
Leu Arg Glu Asn Asn Cys Leu Gln Thr Leu Leu Gln His Leu Lys Ser His Ser 2052

2079  
TTG ACA ATA GTC AGT AAT GCA TGT GGA ACT TTG TGG AAT CTC TCA GCA AGA AAT  
Leu Thr Ile Val Ser Asn Ala Cys Gly Thr Leu Trp Asn Leu Ser Ala Arg Asn 2106

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## FIG. 7H

2133 CCT AAA GAC CAG GAA GCA TTA TGG GAC ATG GGG GCA GTT AGC ATG CTC AAG AAC 2160  
 Pro Lys Asp Gln Glu Ala Leu Trp Asp MET Gly Ala Val Ser MET Leu Lys Asn

2187 CTC ATT CAT TCA AAG CAC AAA ATG ATT GCT ATG GGA AGT GCT GCA GCT TTA AGG 2214  
 Leu Ile His Ser Lys His Lys MET Ile Ala MET Gly Ser Ala Ala Leu Arg

2241 AAT CTC ATG GCA AAT AAG CCT GCG AAG TAC AAG GAT GCC AAT ATT ATG TCT CCT 2268  
 Asn Leu MET Ala Asn Arg Pro Ala Lys Tyr Lys Asp Ala Asn Ile MET Ser Pro

2295 GGC TCA AGC TTG CCA TCT CTT CAT GTT AGG AAA CAA AAA GCC CTA GAA GCA GAA 2322  
 Gly Ser Ser Leu Pro Ser Leu His Val Arg Lys Gln Lys Ala Leu Glu Ala Glu

2349 TTA GAT GCT CAG CAC TTA TCA GAA ACT TTT GAC AAT ATA GAC AAT TTA AGT CCC 2376  
 Leu Asp Ala Gln His Leu Ser Glu Thr Phe Asp Asn Ile Asp Asn Leu Ser Pro

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## FIG. 71

2403  
 AAG GCA TCT CAT CGT AGT AAG CAG AGA CAC AAG CAA AGT AGT CTC TAT GGT GAT TAT  
 Lys Ala Ser His Arg Ser Lys Gln Arg His Lys Gln Ser Leu Tyr Gly Asp Tyr

2430  
 2457  
 GTT TTT GAC ACC AAT CGA CAT GAT GAT AAT AGG TCA GAC AAT TTT AAT ACT ACT GGC  
 Val Phe Asp Thr Asn Arg His Asp Asp Asn Arg Ser Asp Asn Phe Asn Thr Gly

2484  
 2511  
 AAC ATG ACT GTC CTT TCA CCA TAT TTG AAT ACT ACA GTG TTA CCC AGC TCC TCT  
 Asn MET Thr Val Leu Ser Pro Tyr Leu Asn Thr Thr Val Leu Pro Ser Ser Ser

2538  
 2565  
 TCA TCA AGA GGA AGC TTA GAT AGT TCT CGT TCT GAA AAA GAT AGA AGT TTG GAG  
 Ser Ser Arg Gly Ser Leu Asp Ser Ser Arg Ser Glu Lys Asp Arg Ser Leu Glu

2592  
 2619  
 AGA GAA CGC GGA ATT GGT CTA GGC AAC TAC CAT CCA GCA ACA GAA AAT CCA GGA  
 Arg Glu Arg Gly Ile Gly Leu Gly Asn Tyr His Pro Ala Thr Glu Asn Pro Gly

2646  
 2673  
 ACT TCT TCA AAG CGA GGT TTG CAG ATC TCC ACC ACT GCA GCC CAG ATT GCC AAA  
 Thr Ser Ser Lys Arg Gly Leu Gln Ile Ser Thr Thr Ala Ala Gln Ile Ala Lys

2700

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## FIG. 7J

GTC ATG GAA GAA GTG TCA GCC ATT CAT ACC TCT CAG GAA GAC AGA AGT TCT GGG Val MET Glu Glu Val Ser Ala Ile His Thr Ser Gln Glu Asp Arg Ser Ser Gly	2727	2754
TCT ACC ACT GAA TTA CAT TGT GTG ACA GAT GAG AGA AAT GCA CTT AGA AGA AGC Ser Thr Thr Glu Glu Leu His Cys Val Thr Asp Glu Arg Asn Ala Leu Arg Arg Ser	2781	2808
TCT GCT GCC CAT ACA CAT TCA AAC ACT TAC AAT TTC ACT AAG TCG GAA AAT TCA Ser Ala Ala His Thr His Ser Asn Thr Tyr Asn Phe Thr Lys Ser Glu Asn Ser	2835	2862
AAT AGG ACA TGT TCT ATG CCT TAT GCC AAA TTA GAA TAC AAG AGA TCT TCA AAT Asn Arg Thr Cys Ser MET Pro Tyr Ala Lys Leu Glu Tyr Lys Arg Ser Ser Asn	2889	2916
GAT AGT TTA AAT AGT GTC AGT AGT AAT GAT GGT TAT GGT AAA AGA GGT CAA ATG Asp Ser Leu Asn Ser Val Ser Ser Asn Asp Gly Tyr Gly Lys Arg Gly Gln MET	2943	2970

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## FIG. 7K

AAA CCC TCG ATT GAA TCC TAT TCT GAA GAT GAT GAA ACT AAG TTT TGC AGT TAT Lys Pro Ser Ile Glu Ser Tyr Ser Glu Asp Asp Glu Ser Lys Phe Cys Ser Tyr	2997	3024
GGT CAA TAC CCA GCC GAC CTA GCC CAT AAA ATA CAT AGT GCA AAT CAT ATG GAT Gly Gln Tyr Pro Ala Asp Leu Ala His Lys Ile His Ser Ala Asn His MET Asp	3051	3078
GAT AAT GAT GGA GAA CTA GAT ACA CCA ATA AAT TAT AGT CTT AAA TAT TCA GAT Asp Asn Asp Gly Glu Glu Leu Asp Thr Pro Ile Asn Tyr Ser Leu Lys Tyr Ser Asp	3105	3132
GAG CAG TTG AAC TCT GGA AGG CAA AGT CCT TCA CAG AAT GAA AGA TGG GCA AGA Glu Gln Leu Asn Ser Gly Arg Gln Ser Pro Ser Gln Asn Glu Arg Trp Ala Arg	3159	3186
CCC AAA CAC ATA ATA GAA GAT GAA ATA AAA CAA AGT GAG CAA AGA CAA TCA AGG Pro Lys His Ile Ile Glu Asp Glu Ile Lys Gln Ser Glu Gln Arg Gln Ser Arg	3213	3240
AAT CAA AGT ACA ACT TAT CCT GTT TAT ACT GAG AGC ACT GAT GAT AAA CAC CTC Asn Gln Ser Thr Thr Tyr Pro Val Tyr Thr Glu Ser Thr Asp Lys His Leu	3267	3294



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## FIG. 7L

3321  
 AAG TTC CAA CCA CAT TTT GGA CAG CAG GAA TGT GTT TCT CCA TAC AGG TCA CGG  
 Lys Phe Gln Pro His Phe Gly Gln Gln Glu Cys Val Ser Pro Tyr Arg Ser Arg 3348

3375  
 GGA GCC AAT GGT TCA GAA ACA AAT CGA GTG GGT TCT AAT CAT GGA ATT AAT CAA  
 Gly Ala Asn Gly Ser Glu Thr Asn Arg Val Gly Ser Asn His Gly Ile Asn Gln 3402

3429  
 AAT GTA AGC CAG TCT TTG TGT CAA GAA GAT GAC TAT GAA GAT GAT AAG CCT ACC  
 Asn Val Ser Gln Ser Leu Cys Gln Glu Asp Asp Tyr Glu Asp Lys Pro Thr 3456

3483  
 AAT TAT AGT GAA CGT TAC TCT GAA GAA GAA CAG CAT GAA GAA GAA GAG AGA CCA  
 Asn Tyr Ser Glu Arg Tyr Ser Tyr Ser Glu Glu Gln His Glu Glu Glu Arg Pro 3510

3537  
 ACA AAT TAT AGC ATA AAA TAT AAT GAA GAG AAA CGT CAT GTG GAT CAG CCT ATT  
 Thr Asn Tyr Ser Ile Lys Tyr Asn Glu Glu Lys Arg His Val Asp Gln Pro Ile 3564

3591  
 3618

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**FIG. 7M**

GAT TAT AGT TTA AAA TAT GCC ACA GAT ATT CCT TCA CAG AAA CAG TCA TTT  
 Asp Tyr Ser Leu Lys Tyr Ala Thr Asp Ile Pro Ser Ser Gln Lys Gln Ser Phe

3645  
 TCA TTC TCA AAG AGT TCA TCT GGA CAA AGC AGT AAA ACC GAA CAT ATG TCT TCA  
 Ser Phe Ser Lys Ser Ser Gly Gln Ser Ser Lys Thr Glu His MET Ser Ser 3672

3699  
 AGC AGT GAG AAT ACG TCC ACA CCT TCA TCT AAT GCC AAG AGG CAG AAT CAG CTC  
 Ser Ser Glu Asn Thr Ser Thr Pro Ser Ser Asn Ala Lys Arg Gln Asn Gln Leu 3726

3753  
 CAT CCA AGT TCT GCA CAG AGT AGA AGT GGT CAG CCT CAA AAG GCT GCC ACT TGC  
 His Pro Ser Ser Ala Gln Ser Arg Ser Gly Gln Pro Gln Lys Ala Ala Thr Cys 3780

3807  
 AAA GTT TCT TCT ATT AAC CAA GAA ACA ATA CAG ACT TAT TGT GTA GAA GAT ACT  
 Lys Val Ser Ser Ile Asn Gln Glu Thr Ile Gln Thr Tyr Cys Val Glu Asp Thr 3834

3861  
 CCA ATA TGT TTT TCA AGA TGT AGT TCA TTA TCA TCT TTG TCA TCA GCT GAA GAT  
 Pro Ile Cys Phe Ser Ser Arg Cys Ser Ser Leu Ser Ser Leu Ser Ser Ala Glu Asp 3888

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## FIG. 7N

3915  
 GAA ATA GGA TGT AAT CAG ACG ACA CAG GAA GCA GAT TCT GCT AAT ACC CTG CAA 3942  
 Glu Ile Gly Cys Asn Gln Thr Thr Gln Glu Ala Asp Ser Ala Asn Thr Leu Gln

3969  
 ATA GCA GAA ATA AAA GGA AAG ATT GGA ACT AGG TCA GCT GAA GAT CCT GTG AGC 3996  
 Ile Ala Glu Ile Lys Gly Lys Ile Gly Thr Arg Ser Ala Glu Asp Pro Val Ser

4023  
 GAA GTT CCA GCA GTG TCA CAG CAC CCT AGA ACC AAA TCC AGC AGA CTG CAG GGT 4050  
 Glu Val Pro Ala Val Ser Gln His Pro Arg Thr Lys Ser Ser Arg Leu Gln Gly

4077  
 TCT AGT TTA TCT TCA GAA TCA GCC AGG CAC AAA GCT GTT GAA TTT CCT TCA GGA 4104  
 Ser Ser Leu Ser Ser Glu Ser Ala Arg His Lys Ala Val Glu Phe Pro Ser Gly

4131  
 GCG AAA TCT CCC TCC AAA AGT GGT GCT CAG ACA CCC AAA AGT CCA CCT GAA CAC 4158  
 Ala Lys Ser Pro Ser Lys Ser Gly Ala Gln Thr Thr Pro Lys Ser Pro Pro Glu His

4185  
 TAT GTT CAG GAG ACC CCA CTC ATG TTT AGC AGA TGT ACT TCT GTC AGT TCA CTT 4212

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**FIG. 70**

Tyr Val Gln Glu Thr Pro Leu MET Phe Ser Arg Cys Thr Ser Val Ser Ser Leu

4239  
GAT AGT TTT GAG AGT CGT TCG ATT GCC AGC TCC GTT CAG AGT GAA CCA TGC AGT 4266  
Asp Ser Phe Glu Ser Arg Ser Ile Ala Ser Ser Val Gln Ser Glu Pro Cys Ser

4293  
GGA ATG GTA AGT GGC ATT ATA AGC CCC AGT GAT CCA GAT AGC CCT GGA CAA 4320  
Gly MET Val Ser Gly Ile Ile Ser Pro Ser Asp Leu Pro Asp Ser Pro Gly Gln

4347  
ACC ATG CCA CCA AGC AGA AGT AAA ACA CCT CCA CCA CCT CCT CAA ACA GCT CAA 4374  
Thr MET Pro Pro Ser Arg Ser Lys Thr Pro Pro Pro Pro Gln Thr Ala Gln

4401  
ACC AAG CGA GAA GTA CCT AAA AAT AAA GCA CCT ACT GCT GAA AAG AGA GAG AGT 4428  
Thr Lys Arg Glu Val Pro Lys Asn Lys Ala Pro Thr Ala Glu Lys Arg Glu Ser

4455  
GGA CCT AAG CAA GCT GCA GTA AAT GCT GCA GTT CAG AGG GTC CAG GTT CTT CCA 4482  
Gly Pro Lys Gln Ala Ala Val Asn Ala Ala Val Gln Arg Val Gln Val Leu Pro

## FIG. 7P

4509 GAT GCT GAT ACT TTA TTA CAT TTT GCC ACA GAA AGT ACT CCA GAT GGA TTT TCT 4536  
 Asp Ala Asp Thr Leu Leu His Phe Ala Thr Glu Ser Thr Pro Asp Gly Phe Ser

4563 TGT TCA TCC AGC CTG AGT GCT GCT CTG AGC CTC GAT GAG CCA TTT ATA CAG AAA GAT 4590  
 Cys Ser Ser Ser Leu Ser Ala Leu Ser Leu Asp Glu Pro Phe Ile Gln Lys Asp

4617 GTG GAA TTA AGA ATA ATG CCT CCA GTT CAG GAA AAT GAC AAT GGG AAT GAA ACA 4644  
 Val Glu Leu Arg Ile MET Pro Pro Val Gln Glu Asn Asp Asn Gly Asn Glu Thr

4671 GAA TCA GAG CAG CCT AAA GAA TCA AAT GAA AAC CAA GAG AAA GAG GCA GAA AAA 4698  
 Glu Ser Glu Gln Pro Lys Glu Ser Asn Glu Asn Gln Glu Lys Glu Ala Glu Lys

4725 ACT ATT GAT TCT GAA AAG GAC CTA TTA GAT GAT TCA GAT GAT GAT GAT ATT GAA 4752  
 Thr Ile Asp Ser Glu Lys Asp Leu Leu Asp Asp Asp Asp Asp Asp Asp Ile Glu

4779 ATA CTA GAA GAA TGT ATT ATT TCT GCC ATG CCA ACA AAG TCA TCA CGT AAA GGC 4806  
 Ile Leu Glu Glu Cys Ile Ile Ser Ala MET Pro Thr Lys Ser Ser Arg Lys Gly

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**FIG. 7Q**

4833  
 AAA AAG CCA GCC CAG ACT GCT TCA AAA TTA CCT CCA CCT GTG GCA AGG AAA CCA 4860  
 Lys Lys Pro Ala Gln Thr Ala Ser Lys Lys Leu Pro Pro Pro Val Ala Arg Lys Pro

4887  
 AGT CAG CTG CCT GTG TAC AAA CTT CTA CCA TCA CAA AAC AGG TTG CAA CCC CAA 4914  
 Ser Gln Leu Pro Val Tyr Lys Leu Leu Pro Ser Gln Asn Arg Leu Gln Pro Gln

4941  
 AAG CAT GTT AGT TTT ACA CCG GGG GAT GAT ATG CCA CGG GTG TAT TGT GTT GAA 4968  
 Lys His Val Ser Phe Thr Pro Gly Asp Asp MET Pro Arg Val Tyr Cys Val Glu

4995  
 GGG ACA CCT ATA AAC TTT TCC ACA GCT ACA TCT CTA AGT GAT CTA ACA ATC GAA 5022  
 Gly Thr Pro Ile Asn Phe Ser Thr Ala Thr Ser Leu Ser Asp Leu Thr Ile Glu

5049  
 TCC CCT CCA AAT GAG TTA GCT GCT GGA GAA GGA GTT AGA GGA GGA GCA CAG TCA 5076  
 Ser Pro Pro Asn Glu Leu Ala Ala Gly Glu Gly Val Arg Gly Gly Ala Gln Ser

5103  
 GGT GAA TTT GAA AAA CGA GAT ACC ATT CCT ACA GAA GGC AGA AGT ACA GAT GAG 5130  
 Gly Glu Phe Glu Lys Arg Asp Thr Ile Pro Thr Glu Gly Arg Ser Thr Asp Glu

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**FIG. 7R**

GCT CAA GGA GGA AAA ACC TCA TCT GTA ACC ATA CCT GAA TTG GAT GAC AAT AAA Ala Gln Gly Gly Lys Thr Ser Ser Val Thr Ile Pro Glu Leu Asp Asp Asn Lys	5157	5184
GCA GAG GAA GGT GAT ATT CTT GCA GAA TGC ATT AAT TCT GCT ATG CCC AAA GGG Ala Glu Glu Gly Asp Ile Leu Ala Glu Cys Ile Asn Ser Ala MET Pro Lys Gly	5211	5238
AAA AGT CAC AAG CCT TTC CGT TTC GGT AAA AAG ATA ATG GAC CAG GTC CAG CAA GCA Lys Ser His Lys Lys Pro Phe Arg Val Lys Lys Ile MET Asp Gln Val Gln Gln Ala	5265	5292
TCT GCG TCG TCT TCT GCA CCC AAC AAA AAT CAG TTA GAT GGT AAG AAA AAG AAA Ser Ala Ser Ser Ser Ala Pro Asn Lys Asn Gln Leu Asp Gly Lys Lys Lys Lys	5319	5346
CCA ACT TCA CCA GTA AAA CCT ATA CCA CAA AAT ACT GAA TAT AGG ACA CGT GTA Pro Thr Ser Pro Val Lys Pro Ile Pro Gln Asn Thr Glu Tyr Arg Thr Arg Val	5373	5400

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**FIG. 7S**

AGA AAA AAT GCA GAC TCA AAA AAT AAT TTA AAT GCT GAG AGA GTT TTC TCA GAC	5427	5454
Arg Lys Asn Ala Asp Ser Lys Asn Asn Leu Asn Ala Glu Arg Val Phe Ser Asp		
AAC AAA GAT TCA AAG AAA CAG AAT TTG AAA AAT AAT TCC AAG GAC TTC AAT GAT	5481	5508
Asn Lys Asp Ser Lys Lys Gln Asn Leu Lys Asn Asn Ser Lys Asp Phe Asn Asp		
AAG CTC CCA AAT AAT GAA GAT AGA GTC AGA GGA AGT TTT GCT TTT GAT TCA CCT	5535	5562
Lys Leu Pro Asn Asn Glu Asp Arg Val Arg Gly Ser Phe Ala Phe Asp Ser Pro		
CAT CAT TAC ACG CCT ATT GAA GGA ACT CCT TAC TGT TTT TCA CGA AAT GAT TCT	5589	5616
His His Tyr Thr Pro Ile Glu Gly Thr Pro Tyr Cys Phe Ser Arg Asn Asp Ser		
TTG AGT TCT CTA GAT TTT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT	5643	5670
Leu Ser Ser Leu Asp Phe Asp Asp Asp Asp Asp Val Asp Leu Ser Arg Glu Lys Ala		
GAA TTA AGA AAG GCA AAA GAA AAT AAG GAA TCA GAG GCT AAA GTT ACC AGC CAC	5697	5724
Glu Leu Arg Lys Ala Lys Glu Asn Lys Glu Ser Glu Ala Lys Val Thr Ser His		



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## FIG. 7T

5751  
 ACA GAA CTA ACC TCC AAC CAA CAA TCA GCT AAT AAG ACA CAA GCT ATT GCA AAG  
 Thr Glu Leu Thr Ser Ser Asn Gln Gln Ser Ala Asn Lys Thr Gln Ala Ile Ala Lys 5778

5805  
 CAG CCA ATA AAT CGA GGT CAG CCT AAA CCC ATA CTT CAG AAA CAA TCC ACT TTT  
 Gln Pro Ile Asn Arg Gly Gln Pro Lys Pro Ile Leu Gln Lys Gln Ser Thr Phe 5832

5859  
 CCC CAG TCA TCC AAA GAC ATA CCA GAC AGA GGG GCA GCA ACT GAT GAA AAG TTA  
 Pro Gln Ser Ser Lys Asp Ile Pro Asp Arg Gly Ala Ala Thr Asp Glu Lys Leu 5886

5913  
 CAG AAT TTT GCT ATT GAA AAT ACT CCA GTT TGC TTT TCT CAT AAT TCC TCT CTG  
 Gln Asn Phe Ala Ile Glu Asn Thr Pro Val Cys Phe Ser His Asn Ser Ser Leu 5940

5967  
 AGT TCT CTC AGT GAC ATT GAC CAA GAA AAC AAC AAT AAA GAA AAT GAA CCT ATC  
 Ser Ser Leu Ser Asp Ile Asp Gln Glu Asn Asn Asn Lys Glu Asn Glu Pro Ile 5994

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**FIG. 7U**

AAA GAG ACT GAG CCC CCT GAC TCA CAG GGA GAA CCA AGT AAA CCT CAA GCA TCA Lys Glu Thr Glu Pro Pro Asp Ser Gln Gly Glu Pro Ser Lys Pro Gln Ala Ser	6021	6048
GGC TAT GCT CCT AAA TCA TTT CAT GTT GAA GAT ACC CCA GTT TGT TTC TCA AGA Gly Tyr Ala Pro Lys Ser Phe His Val Glu Asp Thr Pro Val Cys Phe Ser Arg	6075	6102
AAC AGT TCT CTC AGT AGT TCT CTT AGT ATT GAC TCT GAA GAT GAC CTG TTG CAG GAA Asn Ser Ser Leu Ser Ser Leu Ser Ile Asp Ser Glu Asp Asp Leu Leu Gln Glu	6129	6156
TGT ATA AGC TCC GCA ATG CCA AAA AAG AAA AAG CCT TCA AGA CTC AAG GGT GAT Cys Ile Ser Ser Ala MET Pro Lys Lys Lys Lys Pro Ser Arg Leu Lys Gly Asp	6183	6210
AAT GAA AAA CAT AGT AGT CCC AGA AAT ATG GGT GGC ATA TTA GGT GAA GAT CTG ACA Asn Glu Lys His Ser Pro Pro Arg Asn MET Gly Gly Ile Leu Gly Glu Asp Leu Thr	6237	6264
CTT GAT TTG AAA GAT ATA CAG AGA CCA GAT TCA GAA CAT GGT CTA TCC CCT GAT Leu Asp Leu Lys Asp Ile Gln Arg Pro Asp Ser Glu His Gly Leu Ser Pro Asp	6291	6318

## FIG. 7V

6345  
TCA GAA AAT TTT GAT TGG AAA GCT ATT CAG GAA GGT GCA AAT TCC ATA GTA AGT  
Ser Glu Asn Phe Asp Trp Lys Ala Ile Gln Glu Gly Ala Asn Ser Ile Val Ser 6372

6399  
AGT TTA CAT CAA GCT GCT GCT GCT GCA TGT TTA TCT AGA CAA GCT TCG TCT GAT  
Ser Leu His Gln Ala Ala Ala Cys Leu Ser Arg Gln Ala Ser Ser Asp 6426

6453  
TCA GAT TCC ATC CTT TCC CTG AAA TCA GGA ATC TCT CTG GGA TCA CCA TTT CAT  
Ser Asp Ser Ile Leu Ser Leu Lys Ser Gly Ile Ser Leu Gly Ser Pro Phe His 35/48 6480

6507  
CTT ACA CCT GAT CAA GAA GAA GAA AAA CCC TTT ACA AGT AAT AAA GGC CCA CGA ATT  
Leu Thr Pro Asp Gln Glu Glu Lys Pro Phe Thr Ser Ser Asn Lys Gly Pro Arg Ile 6534

6561  
CTA AAA CCA GGG GAG AAA AGT ACA TTG GAA ACT AAA AAG ATA GAA TCT GAA AGT  
Leu Lys Pro Gly Glu Lys Ser Thr Leu Glu Thr Lys Lys Ile Glu Ser Glu Ser 6588

6615  
6642

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## FIG. 7W

AAA GGA ATC AAA GGA GGA AAA GGA GGT TAT AAA AGT TTG ATT ACT GGA AAA GTT  
Lys Gly Ile Lys Gly Gly Lys Lys Val Tyr Lys Ser Leu Ile Thr Gly Lys Val

6669  
CGA TCT AAT TCA GAA ATT TCA GGC CAA ATG AAA CAG CCC CTT CAA GCA AAC ATG  
Arg Ser Asn Ser Glu Ile Ser Gly Gln MET Lys Gln Pro Leu Ala Asn MET

6723  
CCT TCA ATC TCT CGA GGC AGG ACA ATG ATT CAT ATT CCA GGA GTT CGA AAT AGC  
Pro Ser Ile Ser Arg Gly Arg Thr MET Ile His Ile Pro Gly Val Arg Asn Ser

6777  
TCC TCA AGT ACA AGT CCT GGT TCT AAA AAA GGC CCA CCC CTT AAG ACT CCA GCC  
Ser Ser Thr Ser Ser Pro Val Ser Lys Lys Gly Pro Pro Leu Lys Thr Pro Ala

6831  
TCC AAA AGC CCT AGT GAA GGT CAA ACA GCC ACC ACT TCT CCT AGA GGA GCC AAG  
Ser Lys Ser Pro Ser Ser Glu Gly Gln Thr Ala Thr Thr Ser Pro Arg Gly Ala Lys

6885  
CCA TCT GTG AAA TCA GAA TTA AGC CCT GTT GCC AGG CAG ACA TCC CAA ATA GGT  
Pro Ser Val Lys Ser Ser Glu Leu Ser Pro Val Ala Arg Gln Thr Ser Gln Ile Gly

## FIG. 7X

6939  
 GGG TCA AGT AAA GCA CCT TCT AGA TCA GGA TCT AGA GAT TCG ACC CCT TCA AGA  
 Gly Ser Ser Lys Ala Pro Ser Arg Ser Gly Ser Arg Asp Ser Thr Pro Ser Arg 6966

6993  
 CCT GCC CAG CAA CCA TTA AGT AGA CCT ATA CAG TCT CCT GGC CGA AAC TCA ATT  
 Pro Ala Gln Gln Pro Leu Ser Arg Pro Ile Gln Ser Pro Gly Arg Asn Ser Ile 7020

7047  
 TCC CCT GGT AGA AAT GGA ATA AGT CCT CCT AAC AAA TTA TCT CAA CTT CCA AGG  
 Ser Pro Gly Arg Asn Gly Ile Ser Pro Pro Asn Lys Leu Ser Gln Leu Pro Arg 7074

7101  
 ACA TCA TCC CCT AGT ACT GCT TCA ACT AAG TCC TCA GGT TCT GGA AAA ATG TCA  
 Thr Ser Ser Pro Ser Thr Ala Ser Thr Lys Ser Ser Gly Ser Gly Lys MET Ser 7128

7155  
 TAT ACA TCT CCA GGT AGA CAG ATG AGC CAA CAG AAC AAC CTT ACC AAA CAA ACA GGT  
 Tyr Thr Ser Pro Gly Arg Gln MET Ser Gln Gln Asn Leu Thr Lys Gln Thr Gly 7182

7209  
 TTA TCC AAG AAT GCC AGT AGT ATT CCA AGA AGT GAG TCT GCC TCC AAA GGA CTA 7236

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**FIG. 7Y**

Leu Ser Lys Asn Ala Ser Ser Ile Pro Arg Ser Glu Ser Ala Ser Lys Gly Leu

7263  
AAT CAG ATG AAT AAT GGT AAT GGA GCC AAT AAA AAG GTA GAA CTT TCT AGA ATG  
Asn Gln MET Asn Asn Gly Asn Gly Ala Asn Lys Lys Val Glu Leu Ser Arg MET

7317  
TCT TCA ACT AAA TCA AGT GGA AGT GAA TCT GAT AGA TCA GAA AGA CCT GTA TTA  
Ser Ser Thr Lys Ser Ser Ser Gly Ser Glu Ser Asp Arg Ser Glu Arg Pro Val Leu

7371  
GTA CGC CAG TCA ACT TTC ATC AAA GAA GCT CCA AGC CCA ACC TTA AGA AGA AAA  
Val Arg Gln Ser Thr Phe Ile Lys Glu Ala Pro Ser Pro Thr Leu Arg Arg Lys

7425  
TTG GAG GAA TCT GCT TCA TTT GAA TCT CTT TCT CCA TCA TCT AGA CCA GCT TCT  
Leu Glu Glu Ser Ala Ser Phe Glu Ser Leu Ser Pro Ser Arg Pro Ala Ser

7479  
CCC ACT AGG TCC CAG GCA CAA ACT CCA GTT TTA AGT CCT TCC CTT CCT GAT ATG  
Pro Thr Arg Ser Gln Ala Gln Thr Pro Val Leu Ser Pro Ser Leu Pro Asp MET

## FIG. 7Z

7533  
TCT CTA TCC ACA CAT TCG TCT GTT CAG GCT GGA TGG CGA AAA CTC CCA CCT  
Ser Leu Ser Thr His Ser Ser Val Gln Ala Gly Trp Arg Lys Leu Pro Pro 7560

7587  
AAT CTC AGT CCC ACT ATA GAG TAT AAT GAT GGA AGA CCA GCA AAG CGC CAT GAT  
Asn Leu Ser Pro Thr Ile Glu Tyr Asn Asp Gly Arg Pro Ala Lys Arg His Asp 7614

7641  
ATT GCA CGG TCT CAT TCT GAA AGT CCT TCT AGA CTT CCA ATC AAT AGG TCA GGA  
Ile Ala Arg Ser His Ser Glu Ser Pro Ser Arg Leu Pro Ile Asn Arg Ser Gly 7668

7695  
ACC TGG AAA CGT GAG CAC AGC AAA CAT TCA TCA TCC CTT CCT CGA GTA AGC ACT  
Thr Trp Lys Arg Glu His Ser Lys His Ser Ser Ser Leu Pro Arg Val Ser Thr 7722

7749  
TGG AGA AGA ACT GGA AGT TCA TCT TCA ATT CTT TCT GCT TCA TCA GAA TCC AGT  
Trp Arg Arg Thr Gly Ser Ser Ser Ser Ile Leu Ser Ala Ser Ser Glu Ser Ser 7776

7803  
GAA AAA GCA AAA AGT GAG GAT GAA AAA CAT GTG AAC TCT ATT TCA GGA ACC AAA  
Glu Lys Ala Lys Ser Glu Asp Glu Lys His Val Asn Ser Ile Ser Gly Thr Lys 7830

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**FIG. 7AA**

7857  
 CAA AGT AAA GAA AAC CAA GTA TCC GCA AAA GGA ACA TGG AGA AAA ATA AAA GAA 7884  
 Gln Ser Lys Glu Asn Gln Val Ser Ala Lys Gly Thr Trp Arg Lys Ile Lys Glu

7911  
 AAT GAA TTT TCT CCC ACA AAT AGT ACT TCT CAG ACC GTT TCC TCA GGT GCT ACA 7938  
 Asn Glu Phe Ser Pro Thr Asn Ser Thr Ser Gln Thr Val Ser Ser Gly Ala Thr

7965  
 AAT GGT GCT GAA TCA AAG ACT CTA ATT TAT CAA ATG GCA CCT GCT GTT TCT AAA 7992  
 Asn Gly Ala Glu Ser Lys Thr Leu Ile Tyr Gln MET Ala Pro Ala Val Ser Lys

8019  
 ACA GAG GAT GTT TGG GTG AGA ATT GAG GAC TGT CCC ATT AAC AAT CCT AGA TCT 8046  
 Thr Glu Asp Val Trp Val Arg Ile Glu Asp Cys Pro Ile Asn Asn Pro Arg Ser

8073  
 GGA AGA TCT CCC ACA GGT AAT ACT CCC CCG GTG ATT GAC AGT GTT TCA GAA AAG 8100  
 Gly Arg Ser Pro Thr Gly Asn Thr Pro Pro Val Ile Asp Ser Val Ser Glu Lys

8127  
 GCA AAT CCA AAC ATT AAA GAT TCA AAA GAT AAT CAG GCA AAA CAA AAT GTG GGT 8154  
 Ala Asn Pro Asn Ile Lys Asp Ser Lys Asp Asn Gln Ala Lys Gln Asn Val Gly

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**FIG. 7BB**

AAT GGC AGT GTT CCC ATG CGT ACC GTG GGT TTG GAA AAT CGC CTG ACC TCC TTT	8181	8208
Asn Gly Ser Val Pro MET Arg Thr Val Gly Leu Glu Asn Arg Leu Thr Ser Phe		
ATT CAG GTG GAT GCC CCT GAC CAA AAA GGA ACT GAG ATA AAA CCA GGA CAA AAT	8235	8262
Ile Gln Val Asp Ala Pro Asp Gln Lys Gly Thr Glu Ile Lys Pro Gly Gln Asn		
AAT CCT GTC CCT GTA TCA GAG ACT AAT GAA AGT CCT ATA GTG GAA CGT ACC CCA	8289	8316
Asn Pro Val Pro Val Ser Glu Thr Asn Glu Ser Pro Ile Val Glu Arg Thr Pro		
TTC AGT TCT AGC AGC TCA AGC AAA CAC AGT TCA CCT AGT GGG ACT GTT GCT GCC	8343	8370
Phe Ser Ser Ser Ser Ser Lys His Ser Ser Ser Pro Ser Gly Thr Val Ala Ala		
AGA GTG ACT CCT TTT AAT TAC AAC CCA AGC CCT AGG AAA AGC AGC GCA GAT AGC	8397	8424
Arg Val Thr Pro Phe Asn Tyr Asn Pro Ser Pro Arg Lys Ser Ser Ala Asp Ser		

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## FIG. 7CC

8451 8478  
 ACT TCA GCT CGG CCA TCT CAG ATC CCA ACT CCA GTG AAT AAC AAC ACA AAG AAG  
 Thr Ser Ala Arg Pro Ser Gln Ile Pro Thr Pro Val Asn Asn Thr Lys Lys

8505 8532  
 CGA GAT TCC AAA ACT GAC AGC ACA GAA TCC AGT GGA ACC CAA AGT CCT AAG CGC  
 Arg Asp Ser Lys Thr Asp Ser Thr Glu Ser Ser Gly Thr Gln Ser Pro Lys Arg

8559  
 CAT TCT GGG TCT TAC CTT GTG ACA TCT GTT TAA  
 His Ser Gly Ser Tyr Leu Val Thr Ser Val .

8570 8580 8590 8600 8610  
 AAGAG AGGAAGAATG AACTAAGAA AATTCTATGT TAATTACAAC

8620 8630 8640 8650 8660 8670 8680  
 TGCTATATAG ACATTTTGT TCAAAATGAAA CTTTAAAGA CTGAAAAATT TTGTAAATAG GTTTGATTCT

8690 8700 8710 8720 8730 8740 8750  
 TGTTAGAGGG TTTTGTCT GGAAGCCATA TTTGATAGTA TACTTTGTCT TCACTGGTCT TATTTGGGA

8760 8770 8780 8790 8800 8810 8820  
 GGCACCTCTG ATGGTTAGGS AAAAAATAGK AAAGCCAAGT ATGTTTGTAAC AGTATGTTTT ACATGTATTT

8830 8840 8850 8860 8870 8880 8890  
 AAAGTAGCAT CCCATCCCAA CTTCCYTTAA TTATTGCTTG TCYTAAATA ATGAACACTA CAGATAGGAA

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# FIG. 7DD

8900 ATATGATATA TTGCTGTTAT CAATCATTTT TAGATTATAA ACTGACTAAA CTTACATCAG GGAATAATTG 8960

8970 GTATTTATGC AAAAAAAA TGTTTTGTG CTTGTGAGTC CATCTAACAT CATAATTAAT CATGTGGCTG 9030

9040 TGAATTCAC AGTAATATGG 9060 9070 9080 9090 9100

9110 AACTGATGGT TCAATTTCAG AAGTAATGAT TAACAGTTAT GTGGTCACAT GATGTGCATA GAGATAGCTA 9170

9180 CAGTGTAATA ATTTACACTA TTTTGTGCTC CAAACAAAAC AAAAATCTGT GTAACTGTAA AACATTGAAT 9240

9250 GAAACTATT TACCTGAAC TACCTGACTC AGATTTTATC TGAAAGTAGG TAGAATTTT GCTATGCTGT AATTGTGTGT 9310

9320 ATATTCTGGT ATTTGAGGTG AGATGGCTGC TCTTTKATTA ATGAGACATG AATTGTGTCT CAACAGAAAC 9380

9390 TAAATGAACA TTTTCAGAATA AATTATTGCT GTATGTAAC TGTTACTGAA ATTGGTATTT GTTTGAAGGG 9450

9460 TSTTGTTTCA CATTGTATT AATTAAATTGT TTAAAAATGCC TCTTTTAAAA GCTTATATAA ATTTTNNCT 9520

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## FIG. 7EE

9530 9540 9550 9560 9570 9580 9590  
TCAGCTTCTA TGCATTAGA GTAAATTC TCTTACTGTA ATAAAAACAR TTGAAGAAGA CTGTTGCCAC

9600 9610 9620 9630 9640 9650 9660  
TTAACCATTC CATGCGTTGG CACTTATCTA TTCCTGAAAT TTCTTTTATG TGATTAGCTC ATCTTGATTT

9670 9680 9690 9700 9710 9720 9730  
TWAAAYATTT TCCACTTAAA CTTTTTTTC TTACTCCACT GGAGCTCAGT AAAAGTAAAT TCATGTAATA

9740 9750 9760 9770 9780 9790 9800  
GCAATGCAAG CAGCCTAGCA CAGACTAAGC ATTGAGCATA ATAGGCCAC ATAATTTCCT CTTTCTTAAT

9810 9820 9830 9840 9850 9860 9870  
AWTATAGAAAT TCTGTACTTG AAATTRATTC TTAGACATTG CAGTCTCTTC GAGGCTTTAC AGTGTAACACT

9880 9890 9900 9910 9920 9930 9940  
GTCTTGCCCC TTCATCTTCT TGTGCAACT GGGTCTGACA TGAACACTTT TTATCACCCCT GTATGTTAGG

9950 9960 9970 9980 9990 10000 10010  
GCAAGATCTC AGCAGTGAAG TATAATCAGC ACTTTGCCAT GCTCANRAAA TTCAAATCAC ATGGAACCTT

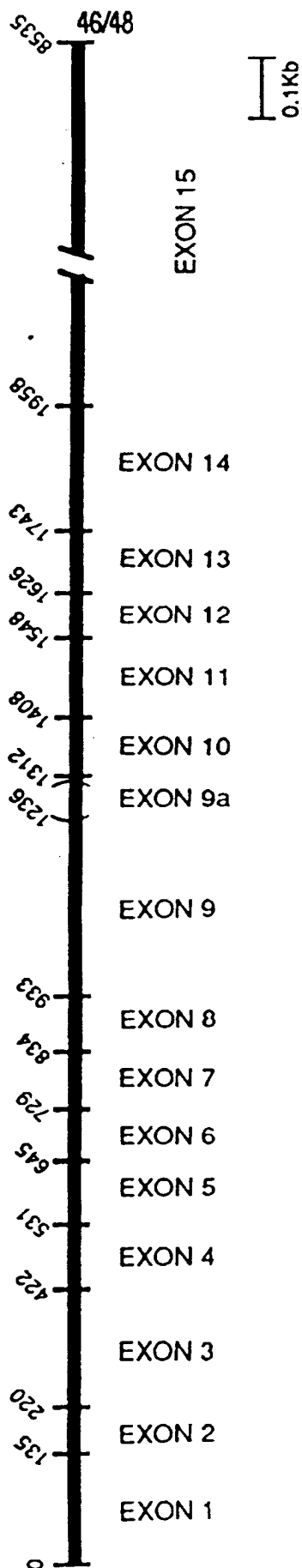
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# FIG. 7FF

10020	10030	10040	10050	10060	10070	10080
AGAGGTAGAT	TTAATACGAT	TAAGATATTC	AGAAGTATAT	TTTAGAATCC	CTGCCCTGTTA	AGGAAACTTT
10090	10100	10110	10120	10130	10140	10150
ATTTGTGGTA	GGTACAGTTC	TGGGGTACAT	GTTAAGTGTC	CCCTTATACA	GTGGAGGGAA	GTCTTCCCTTC
10160	10170	10180	10190	10200	10210	10220
CTGAAGGRAA	ATAAACTGAC	ACTTATTAAAC	TAAAGATAATT	TACTTAATAT	ATCTYCCCTG	ATTTGTTTAA
10230	10240	10250	10260	10270	10280	10290
AAAGATCAGA	GGTGACTGA	TGATACATGC	ATACATATTT	GTTGAATAAA	TGAAAATTTA	TTTTTAGTGA
10300	10310	10320	10330	10340	10350	10360
TAAGANTCAT	ACACTCTGTA	TTTGGGGAGR	GAAAACCTTT	TTAAGCATGG	TGGGGCACTC	AGATAGGNGT
10370	10380					
NAATACACCT	ACCTGGTGGT	CAT				

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**FIG. 8A**



**FIG. 8B-1**

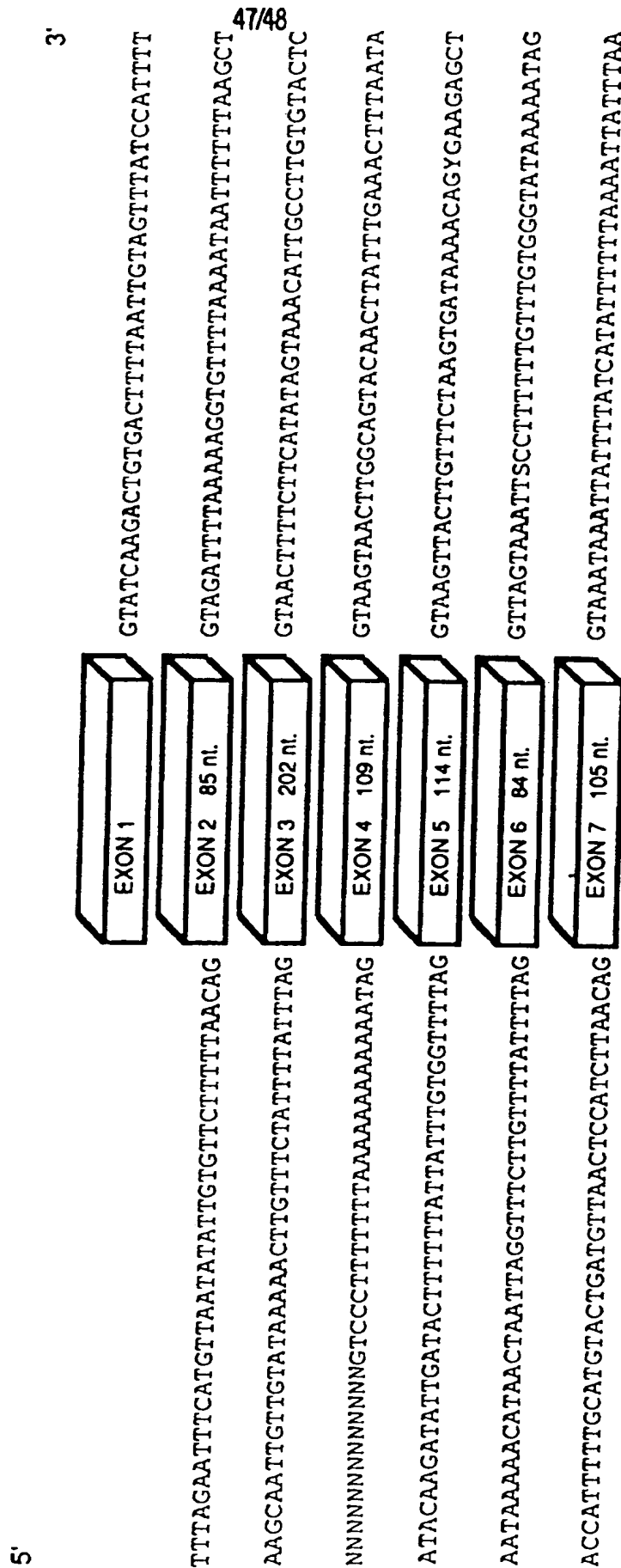


FIG. 8B-2

TAGTCTAAATTATACCATCTATAATGTGCTTAATTTTAG	EXON 8 99 nt.	GTAACAGAAGATTACAAACCCCTGGTCACTAATGCCATGAC
TAAAGTCGTAATTTTGTTTCTAAACTCATTTGGCCCCACAG	EXON 9 379 nt.	GTATGTTCTCTATAGTGACATCGTAGTGCAATGTTTCAAA
ATAACAAGCATTTATGGTTTATGTGATTTTATTTTTCAG	EXON 10 96 nt.	GTAAAGACAAAAATGTTTTTAAATGACATAGACAAATTACTG
TTAGATGATTGTCTTTTTTCCTCTCTGGCCCTTTTAAATTAG	EXON 11 140 nt.	GTATGTTTTTATAACATGTATTTCTTAAAGATAGCTCAGGT
TGNCCTTTAAATGATCCCTCTATTCTGTATTTTAAATTACAG	EXON 12 78 nt.	GTAATATTAGAAATTCACCTGTTTTTCTCTTTTCTCTCTTT
ATTTTATGTATAAAATTAATCTAAAAATTGATTAAATTTCCAG	EXON 13 117 nt.	GTACCTTTGAAAAACATTTAGTACTATAAATGAATTTTCAT
CCAACTCNAATTAGATGACCCCATATTCTGTGTTTCTTACTAG	EXON 14 215 nt.	GTATATATAGAGTTTTTATATTACTTTTAAAGTACAGAATT
ATTGTGACCTTAATTTTGTGATCTCTTGATTTTATTTTCAG	EXON 15	


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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 92/00376

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC Int.Cl. 5 C12Q1/68; C12N15/12		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
Int.Cl. 5	C12Q ; C07K ; G01N	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category <sup>10</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
A	WO,A,8 901 481 (IMPERIAL CANCER RESEARCH TECHNOLOGY) 23 February 1989 see page 2, line 15 - page 6, line 12; claims ---	1
A	BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS. vol. 174, no. 1, 15 January 1991, DULUTH, MINNESOTA US pages 298 - 304; Y.HOSHINO ET AL.: 'Normal human chromosome 5, on which a familial adenomatous polyposis gene is located, has tumor suppressive activity' see abstract see page 302, line 1 - page 303, line 9 ---	26
A	WO,A,9 005 180 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 17 May 1990 see page 7, line 25 - page 9, line 28; claims ---	26-28
-/--		
<p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
01 JUNE 1992	09 JUN 1992	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	LUZZATTO E.R. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	SCIENCE. vol. 253, 9 August 1991, LANCASTER, PA US pages 661 - 665; K.W.KINZLER ET AL.: 'Identification of FAP locus genes from chromosome 5q21' see the whole document ---	1,29,32, 64-66
P,X	SCIENCE. vol. 253, 9 August 1991, LANCASTER, PA US pages 665 - 669; I.NISHISHO ET AL.: 'Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients' see the whole document ---	1,37
T	SCIENCE. vol. 253, 9 August 1991, LANCASTER, PA US page 616; J.MARX: 'Gene identified for inherited cancer susceptibility' ---	

ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.

US 9200376  
SA 57001

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 01/06/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO-A-8901481	23-02-89	EP-A-	0376968	11-07-90
		JP-T-	3503838	29-08-91
		US-A-	5098823	24-03-92
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WO-A-9005180	17-05-90	AU-A-	4635089	28-05-90
		CA-A-	2001815	30-04-90
		EP-A-	0440744	14-08-91
		JP-T-	3505675	12-12-91
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